The University of Chicago

A STUDY OF THE SERUM PROTEINS IN DISEASES OF THE LIVER, WITH SPECIAL REFERENCE TO ELECTROPHORESIS AND THE COLLOIDAL GOLD REACTION

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF MEDICINE
MARCH, 1945

By
SEYMOUR J. GRAY

CHICAGO, ILLINOIS
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PREFACE

This presentation consists of an investigation into the relationship of the liver to the serum proteins, utilizing the electrophoretic technique, and the colloidal gold reaction as methods for the detection of liver disease.

The liver is the principle site of protein storage and synthesis and is intimately associated with the formation of the serum proteins. The electrophoretic method presents an excellent opportunity to study the effects of liver insufficiency upon the serum proteins. The protein abnormalities observed in the serum of patients with hepatic disease may be utilized as a sensitive method of demonstrating liver involvement. The mechanism of the colloidal gold reaction has been investigated, and the clinical application of this method has been studied in a variety of diseases. Although the test depends upon only one of the many functions of the liver, its sensitivity in detecting hepatic disease emphasizes the importance of the liver in the maintenance of normal blood proteins.

The author wishes to express his deep gratitude to Dr. E. S. Guzmán Barrón for his valuable aid and for the use of his laboratory facilities and equipment which made the electrophoretic studies possible. He wishes to acknowledge the helpful suggestions given by Drs. George F. Dick and Walter L. Palmer and the co-operation shown by Dr. Kathryn Knowlton in carrying out many of the chemical determinations.

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PART I. THE COLLOIDAL GOLD REACTION OF BLOOD SERUM IN DISEASES OF THE LIVER

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INTRODUCTION

When a suspension of colloidal gold is added to serial dilutions of spinal fluid, flocculation of the colloidal gold occurs if the protein constituents of the spinal fluid are abnormal. The globulin content (1, 2) and particularly the euglobulin fraction[1] of the spinal fluid are thought to be responsible for the flocculation of the colloidal gold (3).

Many investigators have observed an increase in the serum globulin and a decrease in the serum albumin of patients with liver disease (4). The work of Gros (5), Kendall (6), and de Vries (7) indicates that the increased serum globulin found in hepatic disease differs from that noted in other diseases in that the euglobulin fraction constitutes a higher percentage of the serum globulin content.

Since the flocculation of colloidal gold suspensions may depend upon the globulin or euglobulin fractions the present studies were undertaken to determine the effect of the blood sera of patients with hepatic disease upon the suspension stability of colloidal gold solutions.

REVIEW OF LITERATURE

Zsigmondy (8) laid the foundation for the diagnostic use of the colloidal gold reaction by observing that "certain colloids, especially proteins," prevented the precipitation of colloidal gold suspensions by electrolytes, each protein exerting a specific degree of protection against precipitation. However, Lange (9) later found that proteins within certain dilutions did not prevent but actually caused the precipitations.

The mechanism of colloidal gold flocculation has been the subject of much investigation and will be elaborated upon in Part III. Numerous workers, including Felton (1), Weston (2a),

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[1] The term "euglobulin" is used in the classical sense to designate the least soluble globulin fraction obtained by the earlier fractionation technics. It consists of a mixture of beta and gamma globulins, and will be discussed in Part II.

Cruickshank (2b), and others (2c, d), have shown that the globulin content is the determining factor in the precipitation of colloidal gold and that albumin protects the colloidal suspension from precipitation. The varying colloidal gold curves obtained with spinal fluids in different pathological conditions result from variations in the balance between the precipitating activity of the globulin and the protective action of the albumin.

There is evidence, moreover, that the individual globulin fractions, particularly the euglobulins, play an important role in the precipitation of colloidal gold. Kafka and Sampson (3a) and Haug (3b) concluded that the reaction depends primarily not on the quantitative increase in globulin but on the qualitative alteration in the globulin fractions. Reznikoff (3c) reproduced different colloidal gold curves by varying the relative concentrations of the euglobulin, pseudoglobulin, and albumin. Fischer (3d) expressed the belief that the euglobulin and pseudoglobulin fractions have the strongest precipitating action on colloidal gold solutions. Mellanby and Anwyl-Davies (3e), Reznikoff (3c), and Spiegel-Adolph (3f) observed that the euglobulin fraction exerts a considerable influence on colloidal gold flocculation.

The investigations mentioned heretofore are confined to the flocculation of colloidal gold suspensions by cerebrospinal fluid. It is known, however, that the serum globulin is elevated and the serum albumin is reduced in hepatic disease (4). The present studies of the colloidal gold reaction of blood serum in liver disease were instigated by the observations that alterations in the serum proteins, particularly in the euglobulin fraction (5, 6, 7) are associated frequently with hepatic disease, and by the fact that the euglobulin fraction has been shown to play an important role in colloidal gold flocculation (3).

METHOD OF STUDY

The colloidal gold reaction of the blood serum was studied in 96 cases of diseases of the liver: 46 cases of cirrhosis, 14 of acute parenchymatous disease, 25 of neoplastic involvement, and 11 of miscellaneous disease. The diagnoses were confirmed by autopsy, biopsy, or laparotomy in 11 cases in the first group, 2 in the second, 13 in the third, and 10 in the fourth. A control series and a series of 20 cases of syphilis without clinical evi-

dence of liver disease were studied also. The control series included 300 patients. Two hundred of these were normal adults, and 100 had various extrahepatic diseases. Normal livers were found at autopsy, biopsy, or laparotomy in 70 patients.

In addition to the colloidal gold tests, the chemical studies of the blood included determinations of the plasma albumin, globulin, cholesterol, cholesterol esters, and fibrinogen. The Takata-Ara, bromsulphalein retention, and galactose tolerance tests and other tests of hepatic function were also carried out in many cases as may be noted in Tables 1 to 5.

Preparation of Colloidal Gold

The colloidal gold suspension is prepared in accordance with Patterson's (10) method as used routinely in the serology laboratory of the University of Chicago Clinics: A one per cent solution of potassium oxalate and a one per cent solution of gold chloride dissolved in triple distilled water are titrated with 1/50 normal sodium hydroxide until the characteristic orange-red color is obtained on boiling. The colloidal gold may be prepared in aliquots of 600 cc. and is stable for several months.

Standardization of Colloidal Gold

Whenever a new supply of colloidal gold is made, its acid requirement must be determined. Standardization for this acid requirement depends upon the use of the greatest amount of acid which will give a good control reading for the normal serum and a strongly positive test for the serum of a patient with frank liver disease. The sensitivity of the reaction may be increased or decreased by increasing or decreasing the acidity of the solution. The acid requirement is designated by the "number of cubic centimeters of 1/50 normal hydrochloric acid needed for every 50 cc. of colloidal gold used in performing the tests."

The test is now made on 3 or 4 normal sera using different amounts of 1/50 normal hydrochloric acid for every 50 cc. of colloidal gold used until the ideal acid requirement is attained. This is the greatest amount of acid which may be added to the colloidal gold without producing a false positive reading.

Standardization is done only once for each supply of colloidal gold prepared since several liters may be kept for 2 to 3 months without changing the acid requirement. The required amount

of 1/50 normal hydrochloric acid is then added to the colloidal gold immediately before the test is performed. Usually 1 to 2 cc. of 1/50 normal hydrochloric acid is needed for every 50 cc. of colloidal gold used. The pH of the colloidal gold solution before the addition of acid is approximately 7.6, and the final pH after the addition of the 1/50 normal hydrochloric acid is 7.2.

Blood Serum Colloidal Gold Reaction

Five cubic centimeters of venous blood is obtained from the patient before breakfast and centrifuged; 0.1 cc. of clear serum is removed and diluted 1:350 with a physiologic solution of sodium chloride. In the first of a series of ten tubes is placed 1.8 cc. of a 0.3 per cent solution of sodium chloride. In each of the following nine tubes is placed 1 cc. of a 0.3 per cent solution of sodium chloride. Then 0.2 cc. of the diluted serum is added to the first tube of the series. The contents are mixed, and 1 cc. is then transferred to the second tube and mixed. This procedure is followed throughout the series of ten tubes, and 1 cc. of the mixture in the last tube is discarded. Five cubic centimeters of acidified colloidal gold solution is added to each tube, and the readings are made after twelve to twenty-four hours.

In reading the test the same numbers are used as in reading the regular Lange reaction: 0, red; 1, red-blue; 2, orchid; 3, blue; 4, light blue; 5, colorless.

The greater the precipitation of the colloidal gold, the higher the numbers; 5 represents complete precipitation. The normal range in our experiments varied from 0000000000 to 3332210000. The number 3 appears only occasionally on the left side of the curve. The positive reaction is similar to the dementia paralytica type of curve obtained with spinal fluid--for example, 5532100000 or 5432100000.

RESULTS

Cirrhosis of the Liver

The colloidal gold reaction of the serum was positive in all 46 cases of cirrhosis of the liver. The type and severity of the disease varied. The diagnosis was confirmed by autopsy or biopsy in 11 cases (Table 1).

The plasma proteins were studied in 39 of the 46 cases in

this group. The globulin was increased to an average of 3.19 Gm. per cent (normal range: 1.5 to 2.8 Gm.). Increased values were noted in 23 cases, more frequently in advanced hepatic disease than in early cirrhosis. A decrease in albumin was observed in 26 cases, with an average value of 3.50 Gm. for the group (normal range 4 to 6 Gm.). Low plasma albumin values were associated more closely with the severity and duration of the hepatic disease than with the presence of ascites. Increases in globulin compensated for the decrease in albumin in all but 9 cases, so that the average value of the total plasma proteins was within normal limits (6 to 8 Gm.). Abnormally low or inverted albumin-globulin ratios were found in 28 cases; this fact is in accord with the numerous observations (4) on the frequency of variation of the plasma protein content in hepatic diseases. Although positive colloidal gold reactions were associated in a considerable number of cases with abnormal plasma protein values, they were also obtained for 16 plasmas with normal globulin concentrations, and for 11 with normal albumin-globulin ratios.

The total plasma cholesterol, determined in 39 cases, was normal (140 to 200 mg. per cent) in 20, increased in 7 and decreased in 12 cases in which severe hepatic damage was predominant. The plasma cholesterol esters, determined in 36 cases, were normal (40 to 70 per cent of the total plasma cholesterol) in 14 cases and decreased in 22 cases of extensive involvement of the liver; in 2 of the latter the amount of esters was too low to determine. Better results were obtained with the Takata-Ara test in this group of cases than in any other group in our study, for 28 of the 35 tests performed were positive.

Acute Parenchymatous Hepatic Disease

Positive colloidal gold reactions were obtained in 13 of 14 cases of acute parenchymatous disease of the liver (Table 2). The single negative reaction observed was in the case of a 30-year-old man with the clinical diagnosis of catarrhal jaundice, whose symptoms were subsiding when the test was made (Table 2, Case 5). The plasma proteins were determined in 10 of the 14 cases in this group. The globulin was increased to an average of 3.16 Gm. per cent. It is interesting that there were 6 cases with increased globulin, and that the average globulin values in acute parenchymatous disease of the liver and those in hepatic cirrhosis

approximated each other closely. However, in contrast to the low albumin values found in hepatic cirrhosis, the albumin values in the parenchymatous diseases maintained a normal average of 4.38 Gm. and were abnormally low in only 2 instances. Total plasma proteins were normal in every case, with an average of 7.54 Gm., and were consistently higher than in the cases of cirrhosis of the liver. Positive colloidal gold reactions were again obtained in the presence of both abnormal and normal albumin globulin ratios--4 cases of the former and 6 of the latter.

The changes in the plasma cholesterol and cholesterol esters in this group were somewhat similar to those found in the cases of hepatic cirrhosis. The total plasma cholesterol was determined in 13 of the 14 cases and was found normal in 6, increased in 5, and decreased in 2. The plasma cholesterol esters, determined in 12 cases, were normal in 3 and decreased in 9; in 2 of these the quantity was too small to determine. The Takata-Ara reaction was considerably less sensitive than in the first group of cases, with only 3 positive tests among the 8 carried out.

Neoplastic Involvement of the Liver

There were 25 cases of neoplastic disease of the liver, in which the diagnosis was confirmed in 13 instances by autopsy, biopsy, or laparotomy. Positive colloidal gold reactions were obtained in 19 (Table 3). In 4 of the 6 cases in which the reaction was negative there was minimal hepatic involvement, a few small carcinomatous nodules being found at autopsy. The plasma globulin, which averaged 3.10 Gm. per cent, was increased in 9 cases, whereas the plasma albumin averaged 3.73 Gm. and was decreased in 10 of the 17 cases studied. Since the increase in globulin compensated for the decrease in albumin, the total plasma proteins averaged 6.83 Gm. per cent and were normal in 14 of the 17 cases studied. The degree of abnormality in the value for plasma protein was directly related to the extent and duration of the neoplastic involvement (Table 3, Cases 7, 14, 23, 24). As in the other groups of cases, positive colloidal gold reactions did not occur only with abnormal plasma protein values, but were found in conjunction with normal globulin values in 5 instances and with normal albumin-globulin ratios in 4 instances.

The total cholesterol and the cholesterol esters of the plasma were determined in 16 of the 25 cases. The former was

normal in 7 cases, high in 4, and low in 5 whereas the latter were normal in 5 cases and low in 11 cases of advanced parenchymal damage. The Takata-Ara test was less sensitive here than in the previous group of cases, yielding only 2 positive reactions in the 12 tests performed.

Miscellaneous Hepatic Diseases

The colloidal gold reaction was positive in each of the 11 cases of miscellaneous hepatic diseases, in which the diagnosis was confirmed by autopsy or laparotomy in 10 cases (Table 4). This group included such liver diseases as multiple abscesses, Hodgkin's infiltration, lipoid histiocytosis, extensive fatty degeneration, and miliary infectious granuloma of the liver. The plasma proteins were studied in 9 of the 11 cases. The globulin, averaging 2.99 Gm. per cent, was increased in 5 cases. Although the globulin values were less altered in this group than in the cases of other types of hepatic disease studied, the plasma albumin, averaging 3.27 Gm., was sufficiently low to produce abnormal albumin-globulin ratios in 8 of the 9 cases studied. The total plasma proteins, however, averaged 6.26 Gm. and were within the lower limits of normal in 6 of the 9 cases studied. Here again the colloidal gold reaction was positive in the 4 cases with normal plasma globulin values and in the 1 case with a normal albumin-globulin ratio.

The plasma cholesterol, determined in 9 cases, was normal in 5, high in 2, and low in 2, whereas the cholesterol esters, studied in 7 cases, were normal in 2 and low in 5. In 2 of these cases the values for the cholesterol esters were too low to deter mine. The Takata-Ara reaction was positive in 1 of the 3 tests performed.

Control Studies

The colloidal gold reaction was studied in 200 normal persons with no demonstrable disease of the liver and with no history of previous hepatic involvement. The colloidal gold reaction was negative in the sera of 198 of the 200 healthy individuals. The plasma albumin, globulin, cholesterol, and cholesterol esters were essentially normal in this group.

Extrahepatic Diseases

The colloidal gold reaction was performed in 100 patients with extrahepatic diseases. This series included the acute infectious diseases, acute and chronic glomerulonephritis, nephrosis, extrahepatic jaundice, blood dyscrasias, and neoplasms. The presence of a normal liver was confirmed in 70 of the 100 cases at autopsy, biopsy, or laparotomy. In this group, for example, the liver was found essentially normal at autopsy in chronic glomerulonephritis, Hodgkin's disease, lymphosarcoma, carcinoma of the stomach, pulmonary tuberculosis, and mastoiditis (Table 5). The colloidal gold reaction was negative in the sera of 93 of the 100 patients with extrahepatic diseases (Table 5). Positive reactions were observed in cases of subacute bacterial endocarditis and occasionally in cases of multiple myeloma, lymphopathia venereum, and lupus erythematosis. Data on 50 of these cases of extrahepatic disease are presented in Table 5.

Plasma protein determinations in 75 of the 100 cases of extrahepatic disease revealed a normal average for globulin of 2.80 Gm. per cent and a slightly lowered value for albumin, 3.86 Gm. Normal total proteins, averaging 6.86 Gm. per cent, were present in 65 of the 75 cases. The albumin was diminished in 25 cases; it was markedly decreased in 5 cases of nephrosis and in 4 of chronic nephritis with albuminuria, and was lowered to a lesser degree in 3 cases of heart disease (in 1 of which ascites was present), 4 of infectious diseases, 7 of neoplasm, 1 of hyperthyroidism, and 1 of duodenal ulcer. An increase in the plasma globulin was observed in 22 of the 75 cases studied. Elevated globulin values were observed in chronic glomerulonephritis, nephrosis, subacute bacterial endocarditis, infectious diseases, Boeck's sarcoid, lupus erythematosis, lymphopathia venereum, and multiple myeloma. The colloidal gold reaction was negative in 19 of the 22 cases with high globulin values.

The plasma cholesterol was elevated in 8 cases of extrahepatic jaundice and normal in 2, in contrast to the cholesterol values in intrahepatic jaundice, relatively few of which were above normal. The cholesterol esters were normal in 5 cases of jaundice and lowered in 5. In 65 cases of extrahepatic disease without jaundice, the cholesterol was normal in 48, high in 9, and low in 8, whereas the cholesterol esters were normal in 50 cases and lowered in only 15, in contrast to the predominant lowering

observed in intrahepatic disease.

Serum Colloidal Gold Reaction in Syphilis

The colloidal gold reaction was positive in 8 and negative in 12 of 20 cases of syphilis with no clinical evidence of hepatic involvement. Data for 11 of these cases are given in Table 6. The plasma globulin, averaging 3.17 Gm. per cent, was increased in 5 of the 7 cases in which the proteins were determined, and the colloidal gold reaction was positive in 4 of the 5 cases. The albumin was reduced in 3 of the 7 cases, but the average value was normal--4.13 Gm. In the 6 cases in which the plasma cholesterol and cholesterol esters were determined, the former was normal in 4 and low in 2 while the latter were normal in 3 and low in 3.

COMMENT

In these studies there was a high incidence of positive colloidal gold reactions in the blood sera of patients with hepatic disease. The reactions were positive in 89 of the 96 cases of hepatic disease studied (92.7 per cent). Positive reactions were obtained in every case of cirrhosis of the liver, in all but a single case of acute parenchymatous disease of the liver and in all the cases of miscellaneous hepatic disease studied. The reaction was negative in a case of mild catarrhal jaundice with subsiding symptoms and in 6 cases of neoplastic disease of the liver, in 4 of which autopsy revealed minimal involvement or a few small malignant nodules.

The sensitivity of the colloidal gold reaction was of considerable clinical importance in several cases of hepatic disease, confirmed by autopsy or biopsy in 4 instances, in which bilirubin excretion, bromsulphalein retention, and other tests were negative. In 1 of these cases (L.F. [Table 1, Case 31, a 47-year-old woman with splenomegaly, macrocytic hyperchromic anemia, jaundice, reticulocytosis, and normal blood fragility]), the colloidal gold reaction in the serum was repeatedly positive. Various tests, including the bromsulphalein retention, bilirubin excretion, hippuric acid, galactose tolerance, levulose tolerance, and Takata-Ara tests, gave normal results. A small, firm, diffusely nodular cirrhotic liver was found at operation. Similarly,

normal results were obtained with one or more of the foregoing tests made in 3 cases of hepatic disease in which the diagnoses of portal cirrhosis, primary tumor of the liver, and miliary infectious granuloma of the liver were confirmed at autopsy (Table 1, Case 4; Table 3, Case 12; Table 4, Case 10). The colloidal gold reaction, however, was persistently positive in all 3 cases. It is interesting to note that the bromsulphalein retention test in the 12 cases of hepatic disease in which it was used was positive in 6 cases and negative in 6, while the colloidal gold reaction was positive in 11 of the 12 cases.

Bauer (11) has reported somewhat similar observations on the colloidal gold reaction and compared his results with the Takata-Ara test. In the present series the Takata-Ara test proved less sensitive than the colloidal gold test. It was positive in 28 of 35 cases of hepatic cirrhosis, in 3 of 8 cases of acute parenchymatous liver disease, in 2 of 12 cases of neoplastic hepatic involvement, and in 1 of 3 cases of miscellaneous hepatic disease, a total of 34 positive tests in 58 cases (58.6 per cent).

In cases of extrahepatic jaundice of short duration (such as that resulting from a stone in the common duct) in which the liver was found to be normal at operation, the colloidal gold reactions were negative regardless of the degree of jaundice (Table 5, Cases 1, 2, 3, 5, 6, 7). The amount of serum bilirubin present did not appear to alter the reaction. In cases of extrahepatic jaundice of long duration, however, with secondary liver involvement found at operation, the colloidal gold reaction was positive (Table 4, Case 1). Further studies of liver function in gallbladder disease are presented in Part IV of this thesis.

The reaction was negative in many diseases in which there was no clinically detectable pathologic change in the liver, as in acute and chronic nephritis, infectious diseases, neoplastic diseases, and blood dyscrasias. The diagnosis of a normal liver was confirmed by autopsy, biopsy, or laparotomy in 70 cases. The positive reactions obtained in cases of subacute bacterial endocarditis and passive congestion of the liver were associated with definite evidence of liver disease observed on post mortem examination. The mechanism by which positive colloidal gold reactions may be obtained in occasional instances of multiple myeloma, lymphopathia venereum, and lupus erythematosis will be discussed in Part III.

Observations of the colloidal gold reaction in syphilis suggest a high percentage of disease of the liver following antisyphilitic therapy (Table 6). Five of the 8 positive colloidal gold reactions were observed in patients with histories of previous arsenical therapy; in 1 of these there had been hepatitis (Table 6, Case 7). However, 7 of the 12 negative reactions were found in the sera of patients with histories of similar arsenical therapy. Whether the positive reactions obtained in syphilitic sera represent subthreshold hepatic disease following arsenical therapy (12) or whether they represent false positive reactions, related in some way to the altered globulin content apparently present in syphilis (13), cannot be concluded at this time.

The studies of the plasma cholesterol esters are interesting in that they demonstrate the rather high incidence (66.2 per cent) of abnormal values in hepatic disease (14). Decreases in the cholesterol esters were observed in 47 of 71 cases of hepatic disease; in contrast, in the extrahepatic diseases the esters were decreased in 15 of the 65 cases. The incidence of low cholesterol esters in the various types of hepatic disease was approximately the same: 22 of 36 cases of cirrhosis (61.1 per cent), 9 of 12 cases of acute parenchymatous disease (75 per cent), 11 of 16 cases of neoplastic disease (68.8 per cent), and 5 of 7 cases of miscellaneous hepatic diseases (71.4 per cent). Extensive parenchymal damage was frequently associated with low cholesterol ester values (Table 1, Cases 27, 43; Table 2, Case 14; Table 3, Case 7). Changes in the cholesterol-cholesterol ester ratio paralleled the alteration of the plasma proteins except in the acute parenchymatous diseases, in which decreases in cholesterol esters were found more frequently than altered plasma protein values. The total plasma cholesterol values were not altered so readily, however; they were normal in 38, high in 18, and low in 21 of the 77 cases of hepatic disease studied. Normal values were observed in about half the cases of hepatic disease and in two-thirds of the cases of extrahepatic diseases. An increase in plasma cholesterol was found in 8 of the 10 cases of extrahepatic jaundice, in contrast to the predominantly normal values observed in intrahepatic jaundice.

The poor prognosis associated with low cholesterol ester values (14) is demonstrated in 6 of the 7 cases in which the cholesterol esters were too low to determine. Death occurred in

6 cases within two months; 1 patient (Table 2, Case 7), with catarrhal jaundice, survived. In general, the decrease in cholesterol esters paralleled the severity of the hepatic injury; the cholesterol esters were frequently normal in the early stages of mild hepatic disease.

Variations in the plasma proteins were encountered frequently in 75 cases of hepatic disease in which protein values were determined. The globulin was increased in 43 cases and the albumin decreased in 45. The increase in globulin usually compensated for the decrease in albumin, so that the total plasma proteins were normal in all but 15 cases. It is interesting to observe that the lowest ratios--that is, the greatest increases in globulin and decreases in albumin--were associated with advanced hepatic destruction (Table 1, Case 14; Table 2, Case 14; Table 3, Case 7). The most marked variations in albumin and globulin values were found in cirrhosis of the liver, and the least marked variations were noted in the acute parenchymatous diseases.

The changes in concentration of the plasma proteins were related more closely to the severity and duration of the disease of the liver than to the state of nutrition or to loss of protein in the ascitic fluid. Moderately advanced hepatic disease without demonstrable ascites (Table 3, Cases 7, 23, 24; Table 1, Cases 1, 17, 21, 24) may produce greater variation in protein concentrations than less advanced hepatic disease with ascites (Table 1, Cases 8, 16, 36). One patient with a large, hard, irregular liver, cardiac decompensation, and ascites of long duration had normal plasma proteins (Table 1, Case 41). No definite correlation could be found between malnutrition or inadequate protein intake and altered plasma proteins. Myers and Keefer (4f), Foley and associates (4g), and Snell and Magath (15) have shown that the shifts in plasma proteins so frequently observed in hepatic disease are caused primarily not by deficient intake of protein or mechanical loss in the ascitic fluid but by defective formation of proteins. Holman, Mahoney, and Whipple (16), Kerr, Hurwitz, and Whipple (17), and Sawada (18) and others (19) have concluded that the liver plays an important role in maintaining normal plasma proteins.

The positive colloidal gold reaction does not depend primarily on a quantitative increase in globulin, a decrease in albumin, or an inversion of the albumin-globulin ratio. Positive reactions were present in 29 of the 32 cases with normal plasma

globulin values and in 21 of the 23 cases with normal albumin-globulin ratios. Conversely, in the control series the colloidal gold reaction was negative in 19 of the 22 cases with elevated plasma globulin values and in each of the 25 cases with low or inverted albumin-globulin ratios. The positive colloidal gold reaction may depend, therefore, on a qualitative rather than on a quantitative alteration in the plasma proteins. It is suggested that since euglobulin is believed to play an important role in the colloidal gold precipitation (3) and is thought to be specifically increased in hepatic disease (5, 6, 7), it may be responsible for the positive reactions obtained.

SUMMARY

The colloidal gold reaction of the blood serum was studied in a series of 96 cases of hepatic disease, grouped as follows: 46 cases of cirrhosis, 14 cases of acute parenchymatous disease, 25 cases of neoplastic involvement, and 11 cases of miscellaneous hepatic disease. The diagnoses were confirmed by autopsy, biopsy, or laparotomy in 36 cases: 11 in the first group, 2 in the second, 13 in the third, and 10 in the fourth. The reaction was positive in each of 46 cases of cirrhosis, in 13 of 14 cases of acute parenchymatous involvement, in 19 of 25 cases of neoplasm, and in each of 11 cases of miscellaneous hepatic disease. In the entire series, the reaction was positive in 89 of the 96 cases, or 92.7 per cent.

In several instances the colloidal gold reaction detected liver disease, later confirmed by operation or autopsy, which had not been detected by the usual clinical and chemical tests.

The colloidal gold reaction was negative with sera from 198 of 200 normal adults and in 93 of 100 cases of various extrahepatic diseases; normal livers were found in 70 of these cases at autopsy, biopsy, or laparotomy.

Positive reactions were obtained in 8 of 20 cases of syphilis; in 1 of these 8 cases there was a history of hepatitis but in the others there was no demonstrable hepatic disease. The possibility of latent damage to the liver following arsenical therapy and the question of false positive reactions in syphilis are discussed.

The Takata-Ara reaction was positive in only 34 of 58

cases of hepatic disease (58.6 per cent); it was thus considerably less sensitive than the colloidal gold reaction.

The total plasma cholesterol was normal in 38 of the 77 cases of hepatic disease studied. Decreased values for plasma cholesterol esters, however, were associated frequently with extensive parenchymal damage and were present in 47 of 71 cases of hepatic disease (66.2 per cent). The decrease paralleled the severity of the hepatic injury and was of prognostic significance; 6 of the patients whose cholesterol esters were too low to determine died within two months.

Changes in the cholesterol-cholesterol ester ratio paralleled the shifts in the plasma proteins in all types of liver disease except the acute parenchymatous disease, in which lowered cholesterol esters were more frequent than altered plasma protein values.

Variations in the plasma proteins were frequent in hepatic disease; globulin was increased in 43 cases, and albumin was decreased in 45 of the 75 cases studied. The increase in globulin was usually greater than the decrease in albumin so that the total proteins were normal in 60 of the 75 cases.

The most marked albumin-globulin shifts occurred in cirrhosis of the liver and were associated with advanced hepatic destruction; the least marked variations were found in the acute parenchymatous hepatic diseases. The changes in concentration of the plasma proteins were related more closely to the severity and duration of the hepatic disease than to the state of nutrition or to the loss of protein associated with ascites.

The colloidal gold reaction does not depend primarily upon a quantitative increase in serum globulin or upon a low or inverted albumin-globulin ratio. Positive reactions were obtained in 29 cases of hepatic disease with normal serum globulin determinations and in 21 cases with normal albumin-globulin ratios. The colloidal gold reaction remains unaltered in diseases like nephritis or nephrosis in which the serum globulin is elevated or the albumin-globulin ratio inverted. The mechanism of the reaction is unknown. It is suggested that the serum colloidal gold reaction may depend upon a qualitative rather than upon a quantitative variation in the serum globulin and that the euglobulin may be an important factor in the reaction.

CONCLUSIONS

1. Serial dilutions of the blood serum of patients with liver disease flocculate colloidal gold suspensions producing a paretic type of curve similar to that obtained with spinal fluid.

2. The flocculation of colloidal gold suspensions rarely occurs with the sera of normal individuals and of patients with extrahepatic diseases.

3. The blood serum colloidal gold reaction is a sensitive method of detecting liver disease and may reveal hepatic damage not indicated by other chemical and clinical tests. The sensitivity of the reaction is shown to be of considerable importance by several cases of hepatic disease confirmed by autopsy or biopsy in which other tests of liver function had been negative.

4. Variations in the plasma proteins are observed frequently in hepatic disease and parallel the severity and duration of the liver involvement.

5. The colloidal gold reaction does not depend upon an increase in total serum globulins, a decrease in serum albumin, or an inversion of the albumin-globulin ratio, but may depend upon a qualitative alteration within the globulin fraction of the serum.

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TABLE 1

LIVER FUNCTION STUDIES IN CIRRHOSIS OF THE LIVER

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Fibrinogen, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Wassermann Test	Takata-Ara Test
1	W.Z.	5544531000	3.54/4.21	....	168/79	Neg.	....
2	B.F.	5553310000	.....	....	.....	Neg.	....
3	A.R.	4322000000	2.22/2.97	....	227/..	Neg.	....
4	H.J.	5553210000	3.74/2.29	0.24	150/55	Neg.	Neg.
5	P.M.	5553210000	2.53/4.25	0.21	150/44	Neg.	Pos.
6	A.T.	2552110000	2.80/3.25	0.39	123/33	4+	Pos.
7	H.P.	1553210000	3.31/3.07	....	126/17	Neg.	Pos.
8	J.F.	5555442100	4.46/3.95	0.58	133/38	4+	Pos.
9	A.B.	5532100000	4.22/2.17	0.21	132/..	2+	....
10	F.H.	4453210000	.....	....	208/122	Neg.	Pos.
11	F.M.	5521100000	2.87/2.84	....	138/41	Neg.	Pos.
12	E.S.	5555321000	2.66/3.65	....	176/78	Neg.	Neg.
13	V.S.	5543210000	.....	....	.....	2+	Pos.
14	P.T.	5543100000	2.62/5.06	....	160/70	Neg.	Pos.
15	M.K.	5553210000	3.96/2.53	....	167/too low to measure	Neg.	Pos.
16	B.B.	4322000000	3.01/2.88	....	167/41	Neg.	Pos.
17	J.H.	5542110000	2.97/4.55	....	140/56	Neg.	Pos.
18	M.C.	5321000000	2.49/3.43	....	155/58	Neg.	Neg.
19	A.W.	5453210000	4.12/2.45	....	357/285	Neg.	Pos.
20	P.R.	4533210000	4.02/3.32	....	154/47	Neg.	Pos.
21	L.F.	4455531000	3.42/3.69	....	150/70	Neg.	Pos.
22	A.W.	5453210000	.....	....	.....	....	....
23	P.C.	4421100000	4.15/1.35	....	155/77	Neg.	Pos.
24	S.R.	2533100000	3.68/4.18	....	218/90	Neg.	....

TABLE 1--Continued

Van den Bergh Reaction and Icteric Index	Galactose Tolerance, Bromsulphalein Retention and Other Tests	Diagnosis
Direct--faint de- Indirect--normal	Gal.tol.--1.3 Gm. Brom.--60 per cent	Portal cirrhosis
Icteric index 63	.....	Portal cirrhosis with ascites
.....	.....	Portal cirrhosis (autopsy)
.....	Gal.tol.--2.5 Gm. Brom.--2 per cent Levulose tol.--normal	Portal cirrhosis (autopsy)
Icteric index 17	.....	Portal cirrhosis with ascites
Direct--negative Indirect--normal	.....	Portal cirrhosis with ascites
Icteric index 25	.....	Portal cirrhosis with ascites
Direct--negative Indirect--normal	Brom.--5 per cent	Portal cirrhosis with ascites
Indirect--normal	.....	Portal cirrhosis and syphilis
Icteric index 8.3	.....	Portal cirrhosis
.....	.....	Portal cirrhosis
Indirect--normal	.....	Portal cirrhosis with ascites
Indirect--normal	.....	Portal cirrhosis with ascites
Indirect--normal	.....	Portal cirrhosis (autopsy)
Indirect--7.4 mg. per 100 cc. Icteric index 60	.....	Portal cirrhosis
Indirect--6.2 mg. per 100 cc. Icteric index 50	.....	Portal cirrhosis with ascites
Indirect--normal	.....	Portal cirrhosis
Indirect--normal	.....	Portal cirrhosis
Icteric index 16	Gal.tot.--2.1 Gm.	Portal cirrhosis with esophageal varices
Indirect--normal	.....	Portal cirrhosis
Indirect--1.0 mg. per 100 cc.	.....	Portal cirrhosis
.....	.....	Portal cirrhosis
.....	.....	Portal cirrhosis with esophageal varices
.....	.....	Portal cirrhosis, macrocytic anemia

TABLE 1--Continued

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Fibrinogen, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Wassermann Test	Takata-Ara Test
25	C.W.	1533210000	2.54/3.10	....	156/47	Neg.	....
26	W.W.	4542431000	3.75/3.28	....	162/48	Neg.	Pos.
27	A.V.	5554321000	3.60/1.96	....	125/5.5	Neg.	Pos.
28	J.B.	5555321000	3.03/4.03	0.30	155/63	4+	Pos.
29	P.C.	5555310000	5.09/3.67	....	120/too low to read	Neg.	....
30	M.W.	5554321000	4.38/2.89	....	298/170	Neg.	Neg.
31	L.F.	5553210000	4.76/2.52	0.27	151/60	Neg.	Neg.
32	L.W.	4332100000	3.79/2.81	....	169/95	Neg.	Pos.
33	B.H.	5554210000	.....	....	.....	Neg.	....
34	A.D.	4522100000	4.33/2.80	....	.....	Neg.	Neg.
35	K.R.	5554210000	3.14/3.66	....	137/59	Neg.	Pos.
36	A.A.	5543210000	3.91/2.87	....	160/60	4+	Pos.
37	F.L.	5321000000	.....	....	.....	Neg.	....
38	A.S.	4432100000	3.93/3.02	0.37	147/52	Neg.	....
39	I.B.	5555321000	3.61/2.96	....	120/40	Neg.	Pos.
40	M.S.	5555321000	.....	....	.....	Neg.	Pos.
41	D.L.	1543210000	4.16/2.70	....	160/53	Neg.	Pos.
42	B.J.	5554320000	2.33/5.09	....	138/48	Neg.	Pos.
43	J.P.	5542100000	2.49/3.80	0.23	121/5.4	Neg.	Pos.
44	A.E.	5555310000	4.16/3.18	....	357/80	Neg.	Pos.
45	J.L.	0443210000	2.96/2.60	....	302/..	Neg.	Pos.
46	L.A.	1433100000	4.14/1.65	0.27	88/39	Neg.	Neg.

TABLE 1--Continued

Van den Bergh Reaction and Icteric Index	Galactose Tolerance, Bromsulphalein Retention and Other Tests	Diagnosis
.....	.....	Portal cirrhosis with ascites
Icteric index 53	.....	Portal cirrhosis with ascites
Indirect--0.85 mg. per 100 cc.	.....	Portal cirrhosis
.....	.....	Portal cirrhosis with ascites
.....	.....	Portal cirrhosis
Indirect--normal	.....	Portal cirrhosis (biopsy)
Indirect--4 mg. per 100 cc.	Brom.--no ret. Gal.tol.--2.1 Gm. Benzoic ac.--3.1 Gm.	Early cirrhosis of liver (biopsy), macrocytic anemia, splenomegaly
Indirect--normal	Brom.--12 per cent	Subcapsular cirrhosis (autopsy)
.....	.....	Cardiac cirrhosis; large,hard, pulsating liver; ascites
.....	.....	Cardiac cirrhosis; large,hard, liver; cardiac failure
Indirect--normal	.....	Cardiac cirrhosis; large,hard, nodular liver with ascites
Indirect--normal	.....	Cardiac cirrhosis; tricuspid regurgitation; large,hard, nodular liver with ascites
.....	.....	Cardiac cirrhosis (autopsy)
Direct--neg. Indirect--normal	Gal.tol.--1.0 Gm. Brom.--20 per cent	Cardiac cirrhosis with ascites (biopsy)
.....	.....	Cardiac cirrhosis; tricuspid regurgitation; large,hard, nodular liver
.....	.....	Cardiac cirrhosis; tricuspid regurgitation; large,hard, nodular liver
Indirect--normal	.....	Cardiac cirrhosis; heart failure; large,hard liver with ascites
Indirect--1.1 mg. per 100 cc.	.....	Hemochromatosis with cirrhosis of the liver (autopsy)
Biphasic--5 mg. per 100 cc.	Gal.tot.--3.2 Gm.	Biliary cirrhosis (autopsy)
Icteric index 80	.....	Biliary cirrhosis
Icteric index 82	Gal.tol.--1.5 Gm.	Biliary cirrhosis (autopsy)
Icteric index 15	.....	Atrophic cirrhosis with esophageal varices

TABLE 2

LIVER FUNCTION STUDIES IN ACUTE PARENCHYMATOUS DISEASE OF THE LIVER

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Takata-Ara Test
1	R.S.	5543110000	4.68/2.53	185/67	Neg.
2	P.C.	5543210000	5.81/2.90	232/50	Pos.
3	F.I.	5553210000	4.47/2.22	333/125	Neg.
4	M.H.	5532110000	.....	154/33.6	Pos.
5	F.W.	0211000000	.....	.....	....
6	F.G.	5554000000	.....	131/..	...
7	L.K.	5542210000	.....	181/too low to measure	Pos.
8	F.B.	5532100000	4.23/3.19	177/92	....
9	F.P.	5555421000	3.74/3.75	161/63	....
		2332110000	4.37/2.01	.....	....
10	R.S.	4432110000	4.19/2.84	212/87	....
11	A.P.	4543100000	3.97/5.03	201/41.2	Neg.
12	L.B.	4533210000	4.33/3.35	241/65	Neg.
13	P.V.	4444310000	5.16/1.87	238/90	Neg.
14	A.N.	5555431000	3.28/5.04	119/not readable	....

TABLE 2--Continued

Van den Bergh Reaction and Icteric Index	Galactose Tolerance, Bromsulphalein Retention and Other Tests	Diagnosis
Icteric index 40	Gal.tol.--5.6 Gm.	Catarrhal jaundice
Icteric index 83	Gal.tol.--4.4 Gm.	Catarrhal jaundice
Icteric index 46	.....	Catarrhal jaundice
Icteric index 30	.....	Catarrhal jaundice
.....	.....	Catarrhal jaundice
Icteric index 37	.....	Catarrhal jaundice
Direct--strong biphasic	.....	Catarrhal jaundice
Indirect--4 mg. per 100 cc.	.....	Catarrhal jaundice
Indirect--10 mg. per 100 cc.	Brom.--10 per cent	Infectious hepatitis (autopsy)
Icteric index 75	.....	Toxic hepatitis with jaundice following pneumonia
Icteric index 15	.....	Six weeks later, no symptoms
Icteric index 60	Gal.tol.--3.0 Gm.	Arsenical hepatitis
Icteric Index 65	Gal.tol.--6.4 Gm.	Arsenical hepatitis
Icteric index 50	.....	Arsenical hepatitis
Icteric index 49	Benzoic acid--1.62 Gm.	Cinchophen poisoning of liver
Direct--immediate; indirect--10 mg. per 100 cc.	.....	Infectious hepatitis (autopsy)

TABLE 3

LIVER FUNCTION STUDIES IN NEOPLASTIC DISEASES OF THE LIVER

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Fibrinogen, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Takata-Ara Test
1	L.M.	4432110000	3.59/2.44	0.31	140/49	Neg.
2	S.U.	4555320000	4.50/2.72	....	172/70	...
3	P.M.	0233210000	4.24/2.49	....	127/63	Neg.
4	F.F.	5532110000	.....	....	.....	....
5	P.R.	0000100000	3.49/2.62	....	136/62	....
6	J.S.	1543210000	5.52/2.86	....	212/58	Neg.
7	B.K.	5543210000	1.76/4.73	....	58/not readable	Neg.
8	C.R.	0253310000	.....	....	.....	....
9	E.S.	5553210000	.....	....	.....	Pos.
10	M.J.	5321000000	4.26/2.44	....	139/38	Neg.
11	N.S.	0042210000	.....	....	.....	Neg.
12	A.M.	0454321000	3.55/3.07	0.6	185/73	Neg.
13	I.D.	5532100000	.....	....	.....	....
14	P.P.	5555321000	2.56/3.98	....	160/40	....
15	C.P.	1155321000	.....	....	.....	Neg.
16	D.L.	5553210000	3.94/2.71	0.87	239/98	....
17	H.A.	5555431000	6.20/3.50	....	118/42	Pos.
18	J.W.	5532100000	.....	....	.....	Neg.
19	A.K.	0211100000	2.21/3.25	....	.....	....
20	S.H.	1333210000	.....	....	.....	....
21	A.V.	2332210000	3.31/3.53	1.35	111/32	....
22	B.O.	0123211000	4.56/1.49	....	212/46	....
23	J.L.	4543210000	3.50/4.31	....	180/54	Neg.
24	F.P.	5533211000	2.06/3.48	0.25	141/44	....
25	P.G.	0532210000	4.19/3.15	....	227/44	....

TABLE 3--Continued

Van den Bergh Reaction and Icteric Index	Primary Source of Tumor
Indirect--normal	Carcinoma of stomach with metastases to liver (laparotomy)
.....	Carcinoma of stomach with metastases to liver (laparotomy)
Indirect--normal	Carcinoma of stomach with a few small metastatic nodules in liver (laparotomy)
.....	Carcinoma of stomach with metastases to liver (autopsy)
.....	Carcinoma of stomach with metastases to liver (laparotomy); bromsulph. test normal
Icteric index 99	Carcinoma of stomach with metastases to liver
Direct--positive; indirect--4.7 mg. per 100 cc.	Carcinoma of stomach with metastases to liver (autopsy)
.....	Carcinoma of breast with metastases to liver (autopsy)
Indirect--normal	Carcinoma of breast with metastases to liver
Indirect--4.6 mg. per 100 cc.	Carcinoma of breast with metastases to liver
.....	Primary carcinoma of liver (biopsy)
Indirect--normal	Primary tumor of liver (autopsy)
.....	Carcinoma of prostate with metastases to liver, and ascites
.....	Carcinoma of prostate with metastases to liver, and ascites
Indirect--normal	Carcinoma of rectum with metastases to liver, and ascites
Indirect--normal	Carcinoma of prostate with metastases to liver (biopsy)
Indirect--normal	Carcinomatosis with involvement of liver
.....	Carcinoma of pancreas with metastases to liver
Indirect--5.4 mg. per 100 cc.	Carcinoma of biliary tract with small amount of infiltration at hilus of liver (autopsy)
Direct--biphasic; indirect--8.5 mg. per 100 cc.	Carcinoma of pancreas with a few small nodules in liver (autopsy)
.....	Carcinoma of pancreas with metastases to liver
.....	Carcinoma of pancreas extending up into hilus of liver (laparotomy); gal. tol., 5.8 Gm.
Icteric index 60	Carcinoma of rectum with metastases to liver
Indirect--normal	Carcinoma of rectum with metastases to liver
Biphasic--strong; indirect--8.3 mg. per 100 cc.	Carcinoma of rectum with metastases to liver; gal. tol., 5.7 Gm.

TABLE 4

LIVER FUNCTION STUDIES IN MISCELLANEOUS DISEASES OF THE LIVER

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Fibrinogen, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Takata-Ara Test
1	O.Y.	5553221000	4.98/2.41	....	258/143	Pos.
2	A.H.	4543210000	2.06/2.40	0.55	106/too low to measure	....
3	R.C.	2433210000	3.31/3.20	....	176/65	....
4	B.H.	4532100000	1.93/2.40	....	.....	....
5	E.K.	5521000000	3.05/2.38	....	120/too low to measure	....
6	C.D.	5555421000	2.73/3.68	....	139/..	....
7	B.K.	1531000000	3.69/3.01	....	180/60	....
8	G.B.	4522110000	.....	....	.....	Neg.
9	S.K.	5532110000	4.33/3.33	....	160/58	....
10	B.L.	5553210000	3.42/4.12	0.65	169/67	...
11	W.W.	5422210000	.....	...	376/..	Neg.

TABLE 4--Continued

Van den Bergh and Icteric Index	Galactose Tolerance, Bromsulphalein Retention and Other Tests	Diagnosis
Icteric index 62	.....	Chronic cholecystitis with hepatitis (laparotomy)
.....	Brom.--20 per cent	Abscesses of liver (laparotomy)
.....	.....	Hodgkin's disease--infiltration of liver (autopsy)
.....	.....	Extreme degree of fatty degeneration of liver (autopsy)
Indirect--10 mg. per 100 cc.	.....	Considerable fatty degeneration of liver (autopsy)
.....	.....	Considerable fatty degeneration of liver (autopsy)
.....	.....	Marked fatty degeneration of liver (autopsy)
.....	Gal.tol.--2.7 Gm.; brom.--no ret.	Fatty infiltration of liver following diabetes (autopsy)
.....	Gal.tol.--0.5 Gm.; brom.--no ret.	Hepatosplenomegaly and anemia
.....	Brom.--3 per cent; benzoic acid--2.1 Gm.	Miliary infectious granuloma of liver (autopsy)
.....	Gal.tol.--2 Gm.	Lipoid histiocytosis (autopsy)

TABLE 5

LIVER FUNCTION STUDIES IN VARIOUS EXTRAHEPATIC DISEASES

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Icteric Index	Diagnosis
1	J.O.	0001200000	.....	212/46	28	Common duct stone; liver normal (laparotomy)
2	G.W.	0123100000	5.57/3.14	313/163	125	Common duct stone; liver normal at operation
3	E.B.	0210000000	4.36/2.82	208/49	75	Common duct stone; liver normal at operation
4	L.M.	0110000000	5.05/2.89	170/71	...	Cholelithiasis; liver normal at operation
5	C.A.	0121000000	4.75/2.26	.....	115	Common duct stone; liver normal at operation
6	F.S.	0001000000	3.96/2.53	202/87	30	Common duct stone; liver normal at operation
7	T.H.	0013210000	4.11/2.86	193/60	41	Cholelithiasis; liver normal at operation
8	N.P.	0211000000	1.90/2.62	.....	...	Nephrosis
9	A.B.	2221000000	1.19/2.73	444/250	...	Nephrosis
10	A.M.	0111100000	1.90/3.20	.....	...	Nephrosis
11	D.H.	1211000000	1.63/3.31	.....	...	Chronic glomerulonephritis
12	A.L.	0000000000	2.90/3.87	.....	...	Chronic glomerulonephritis; liver normal at autopsy
13	S.K.	1121000000	4.01/3.33	175/86	...	Chronic glomerulonephritis
14	A.S.	2221000000	2.96/3.41	.....	...	Chronic glomerulonephritis
15	F.G.	0111100000	4.75/3.14	174/90	...	Chronic glomerulonephritis; liver normal at autopsy
16	E.F.	2321000000	3.20/1.89	.....	...	Chronic glomerulonephritis; liver normal at autopsy
17	N.G.	1111000000	1.32/2.59	265/118	...	Chronic glomerulonephritis; liver normal at autopsy
18	A.M.	0110000000	3.80/2.72	176/29	...	Hodgkin's disease; liver normal at autopsy
19	B.G.	0111100000	4.29/1.93	135/54	...	Hodgkin's disease
20	K.R.	0110000000	4.22/1.81	.....	...	Lymphosarcoma (liver normal at autopsy)
21	G.S.	0110000000	.....	.....	...	Carcinoma of stomach; pneumonia; liver normal at autopsy
22	J.T.	2200000000	3.82/2.52	192/81	...	Carcinoma of stomach; liver normal at autopsy
23	A.L.	0022100000	3.54/3.12	171/51	...	Kidney tumor; liver normal at autopsy
24	B.C.	1123210000	3.98/3.58	.....	...	Carcinoma of lung

TABLE 5--Continued

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Icteric Index	Diagnosis
25	W.D.	0122100000	4.84/2.89	177/51	55	Carcinoma of biliary tract; liver normal at operation
26	A.B.	5554310000	3.41/3.53	129/64	...	Subacute bacterial endocarditis
27	C.J.	2221000000	4.48/3.05	.....	...	Subacute bacterial endocarditis
28	A.S.	0001200000	3.86/3.06	184/83	...	Coronary occlusion
29	A.H.	5553210000	2.89/3.34	182/102	...	Passive congestion of liver from cardiac failure (autopsy)
30	B.T.	0120000000	4.56/2.46	178/29	...	Rheumatic heart disease
31	S.S.	0111100000	2.96/3.46	160/30	...	Erysipelas
32	O.E.	0121100000	4.02/3.61	134/53	...	Acute sinusitis
33	S.L.	1232100000	3.14/3.24	.....	...	Mastoiditis; temperature 104 F.; liver normal at autopsy
34	A.F.	0110000000	.....	.....	...	Pulmonary tuberculosis; liver normal at autopsy
35	S.W.	0121100000	5.43/1.28	159/67	...	Pulmonary tuberculosis; liver normal at autopsy
36	A.L.	0110000000	4.66/2.34	.....	...	Pernicious anemia
37	B.G.	0221000000	4.87/2.28	141/55	...	Pernicious anemia
38	S.R.	0210000000	5.45/2.04	.....	...	Duodenal ulcer
39	P.N.	1123200000	2.98/4.02	206/108	...	Duodenal ulcer
40	R.C.	0110000000	4.50/2.06	190/88	...	Spastic colitis
41	S.H.	1221100000	4.29/2.17	174/69	...	Bacillary dysentery
42	S.H.	0111000000	3.54/2.17	128/49	...	Hyperthyroidism
43	L.G.	1100000000	5.16/1.88	143/62	...	Splenomegaly; liver normal (biopsy)
44	S.H.	0121000000	4.87/2.88	187/98	...	Boeck's sarcoid
45	G.A.	0111000000	4.35/2.21	.....	...	Multiple myeloma
46	F.B.	2211000000	5.42/3.10	.....	...	Multiple myeloma
47	G.L.	2221100000	4.10/2.61	180/78	...	Lupus erythematosis
48	F.B.	5211000000	3.76/3.18	.....	...	Lupus erythematosis
49	S.J.	5221000000	4.21/2.76	175/64	...	Lymphopathia venereum
50	B.S.	2211100000	4.15/3.14	.....	...	Lymphopathia venereum

TABLE 6

LIVER FUNCTION STUDIES IN SYPHILIS

Case No.	Patient	Serum Colloidal Gold Reaction	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Chlosterol/ Chlosterol Esters, Mg. Per Cent	Wassermann Reaction	Diagnosis
1	F.M.	1221100000	4.07/2.17	156/76	3+	Central nervous system syphilis; no arsenical therapy
2	F.K.	5442110000	3.69/3.46	162/42	4+	Central nervous system syphilis; arsenical therapy
3	A.S.	5555321000	4.76/2.89	134/58	4+	Central nervous system syphilis; arsenical therapy
4	L.S.	2532110000	3.68/3.44	159/60	4+	Syphilitic heart disease; arsenical therapy
5	T.M.	5521110000	4.17/3.13	.....	4+	Syphilitic heart disease; no arsenical therapy
6	B.S.	1532100000	.....	.....	...	Syphilitic heart disease; no arsenical therapy
7	N.M.	5552100000	4.85/3.14	175/90	3+	Primary syphilis; mild hepatitis from arsenical therapy
8	S.L.	5321000000	.....	.....	...	Primary syphilis; arsenical therapy
9	L.F.	1221000000	.....	.....	...	Secondary syphilis; arsenical therapy
10	F.M.	5521000000	.....	.....	...	Secondary syphilis; arsenical therapy
11	N.D.	0231000000	3.71/4.02	127/44	4+	Tertiary syphilis; splenomegaly; liver normal size

PART II. THE ELECTROPHORETIC ANALYSES OF THE SERUM PROTEINS IN DISEASES OF THE LIVER

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INTRODUCTION

The investigations of Gros (1), Kendall (2), and de Vries (3) indicate that the euglobulin content of the blood serum, determined by fractional precipitation, is elevated specifically in hepatic disease. Tiselius (4) has emphasized the limitations of protein analysis by fractional precipitation. In view of the great tendency towards coprecipitation, the globulin subfractions cannot be measured accurately when determined by the salting out procedures. Tiselius submitted the euglobulin and other globulin fractions obtained by fractional precipitation to electrophoretic analysis and found that they were mixtures of all the proteins with the predominance of one or more fractions. Euglobulin, for example, yielded beta and gamma globulin upon electrophoretic analysis. It is generally recognized that the electrophoretic method is the most accurate means of measuring and analyzing the protein fractions.

In view of the fact that the serum colloidal gold reaction is positive in the acute parenchymatous diseases of the liver in which the protein fractions, determined by fractional precipitation, appear to be normal, it was concluded in the previous section that the reaction is not related primarily to a quantitative increase in globulin, but rather to some qualitative alteration within the globulin fractions of the serum.

There is little information available concerning the electrophoretic distribution of the serum proteins in different types of hepatic disease and in various stages of liver insufficiency, particularly in the early stages of liver disease in which the serum proteins are presumably normal by chemical analysis.

In order to elucidate further upon the relationship of hepatic disease to the serum proteins and to study the mechanism of the blood serum colloidal gold reaction in diseases of the liver, the sera of patients with various types of liver diseases were subjected to electrophoretic analysis.

The present investigation includes the electrophoretic analysis of the serum proteins of patients with cirrhosis of the liver, acute parenchymatous hepatic diseases, and cancer of the

liver. Patients with extrahepatic jaundice caused by gall stones were studied likewise, and the results obtained by electrophoretic and chemical analysis in the different types of liver disease and in extrahepatic jaundice were compared. Patients with varying degrees of liver insufficiency were studied in each group.

METHOD OF STUDY

The blood samples were obtained from the patients before breakfast. The serum was diluted with 3 parts of veronal buffer made by mixture of the required amounts of 0.025 M veronal, 0.025 M hydrochloric acid, and 0.025 M sodium chloride at pH 7.8 at 25°C., and dialyzed against several liters of this buffer for 3 to 4 days at 3°C., the buffer being changed daily. The protein solution was then centrifuged at 3°C. before being introduced into the electrophoresis cell.

The electrophoretic experiments were carried out in the Tiselius apparatus (4) at 3°C., the electrophoretic patterns being recorded by the method described by Longsworth (5).

The Tiselius cell consists of four compartments which can be disconnected and connected at will. The protein solution was introduced into the two lower compartments of the cell, which were then disconnected from the upper two cells. The buffer against which the protein had been dialyzed was added to the two upper compartments, and the whole cell was immersed in a constant temperature bath at 3°C. After allowing twenty minutes for temperature equilibrium the two upper cells containing the buffer were made continuous with the two lower cells containing the protein and the direct current of six milliamperes was applied to the protein solution.

The different protein fractions separate as distinct boundaries because of their difference in electrophoretic mobility under the conditions of pH and ionic strength used. The concentration of protein at each protein buffer boundary alters the refractive index at that boundary, which may be measured by the downward deflection of a beam of light passing through the boundary. These deflected rays of light are intercepted by a moving schlieren diaphragm and are recorded for each protein boundary by the automatic camera developed by Philipot (6) and Svensson (7).

The resulting diagram consists of a number of peaks each

corresponding to a protein boundary (Fig. 1). The area under each peak may be measured and is proportional to the concentration of the protein fraction which that boundary represents.

The concentrations of the components of serum protein were estimated from the electrophoretic diagrams obtained from the descending boundaries.

RESULTS

Normal Serum

The 4 protein fractions of human serum demonstrable by this method, as first noted by Tiselius (4) and Stenhagen (8), are albumin and alpha, beta, and gamma globulin, in the order of their electrophoretic mobility. There is excellent agreement among various workers concerning the proportions of these fractions in normal serum. In the 5 normal patients studied here (Fig. 1), the serum albumin constituted 62.5 to 65.5 per cent of the total protein, alpha globulin, 6.2 to 7.9 per cent, beta globulin, 12.6 to 15.2 per cent, and gamma globulin, 13.1 to 15.7 per cent (Table 1). The average normal values of 64 per cent for serum albumin and 7.0 per cent, 14.5 per cent, and 14.4 per cent for the alpha, beta, and gamma globulins, respectively, closely approximate those reported by Svensson (7), Longsworth (5), Luetscher (9), Gutman (10), and Kekwick (11) (Table 2). The high value for gamma globulin reported by Kekwick is caused by the "delta effect," probably due to diffusion, observed in the ascending boundary. All of our studies were made in the descending boundary to obviate this effect, as suggested by Longsworth and others.

Cirrhosis of the Liver

Electrophoretic studies of the serum proteins were made in 12 patients with cirrhosis of the liver (Fig. 4). The diagnosis was verified in 8 of these cases by autopsy, biopsy, or peritoneoscopy. Atrophic cirrhosis of the liver was found in 6 cases, hypertrophic periportal cirrhosis in 2, polyserositis with cirrhosis of the liver in 1, and pellagra with atrophic cirrhosis in 1. These patients were all severely jaundiced with icteric indices between 30 and 120.

The abnormality of the serum proteins is more pronounced in cirrhosis of the liver than in any other form of liver disease. A decrease in serum albumin or increase in serum globulin, determined chemically, was observed in 9 of 10 cases (Table 3). Electrophoretic studies demonstrate even more severe alteration of the serum proteins. Serum albumin was decreased to an average of 45.7 per cent and varied from 31.6 to 64.5 per cent. These determinations were only slightly lower in the 6 cases with ascites than in the 6 without ascites, the average for the former group being 44.7 per cent and for the latter, 46.8 per cent. As many albumin determinations of 31.6 to 39.9 per cent were observed in patients without ascites as in those with ascites, although the 2 normal values of 64.3 and 64.5 per cent occurred in patients without ascites.

The most consistent and characteristic globulin alteration is a large increase in the gamma globulin. This was observed in 11 of the 12 cases studied. The gamma globulin was increased to an average of 29.3 per cent, a 100 per cent increase over the normal. Determinations as high as 36.2, 39.2, 44.4, and 49.0 per cent were observed in this group.

Although the beta globulin was increased in 5 of the 12 patients studied, high blood cholesterol values were found in 2 of these cases (Table 3, Cases 15 and 62). In the remaining 3 cases in which the blood cholesterol and fat were normal, the beta globulin constituted 18.4 to 22.5 per cent of the total protein. In general, the average beta globulin for the 10 patients with normal blood cholesterol and fat determinations was normal (14.6 per cent), although moderate increases in the beta globulin were noted in several cases (Table 3, Cases 7, 20, 25).

Abnormally high values of 9.8 per cent or more for alpha globulin were observed in 5 cases in which the albumin determinations were particularly low (31.6, 38.1, 39.9, 42.7 per cent), and where there was an inadequate compensatory increase in beta and gamma globulin. In other instances with equally low serum albumin and normal alpha globulin values, there were unusually large compensatory increases in beta or gamma globulin (Table 3, Cases 22, 61).

Acute Parenchymatous Liver Disease

The serum proteins of 5 patients with catarrhal jaundice,

and 1 with an acute arsenical hepatitis, were studied electrophoretically (Table 4). The catarrhal jaundice patients were between the ages of 5 and 25 and were rather severely jaundiced, with icteric indices between 21 and 40. They presented the typical history and course of an acute infectious hepatitis; biopsy confirmed the diagnosis in 1 patient with catarrhal jaundice (Table 4, Case 29), and in one with acute arsenical hepatitis (Table 4, Case 14).

Although the total serum globulin determined chemically by the method of Campbell and Hanna (12) was normal in 3 of the 5 patients with catarrhal jaundice (Table 4, Cases 32, 42, 68), electrophoretic analysis revealed an abnormal increase in beta or gamma globulin or both in every instance (Fig. 2). The increase of beta globulin to 20.5 and 25.9 per cent noted in 2 patients (Table 4, Cases 29 and 33) may be considered valid, since the blood cholesterol and the total fats which migrate with beta globulin were normal.

A considerable increase in gamma globulin, with values ranging between 22.2 and 44.2 per cent, was observed in 4 of the 5 cases of catarrhal jaundice. This increase in the gamma globulin to an average of 25.9 per cent and the concomitant decrease in serum albumin are the characteristic protein changes in the acute parenchymatous liver diseases.

That these protein abnormalities may be present in spite of a normal albumin-globulin ratio, determined chemically, is well illustrated in the case of the 5-year-old child with catarrhal jaundice (Table 4, Case 32). The albumin was decreased to 40.3 per cent and the gamma globulin increased to 3 times its normal value, although the albumin-globulin ratio obtained by the usual fractional precipitation methods remained normal (Fig. 3).

Figure 3 also demonstrates the fact that the ascending and descending boundaries are not mirror images. A fifth protein appears between the beta and gamma globulin in the anode, and the "delta effect" described by Longsworth may be seen in the very large gamma globulin of the ascending boundary. In the descending boundary, however, the additional protein appears to be a component of the gamma globulin, and the "delta effect" is not observed.

An increase in both beta and gamma globulin was observed in the serum of the patient with acute arsenical hepatitis (Table 4,

Case 14). This 18-year-old patient became severely jaundiced following the third of a series of arsenical injections. The icteric index rose to 150, and the patient presented the symptoms of complete obstructive jaundice. At operation, no extrahepatic disease was found, and a biopsy of the liver revealed a severe hepatitis with intrahepatic obstruction, characterized by bile casts in the hepatic bile capillaries and edema of the periportal tissue.

Several chemical determinations of the serum proteins revealed a normal albumin-globulin ratio. The serum proteins were abnormal by electrophoretic analysis, however, the albumin being reduced to 44.5 per cent and the gamma globulin increased to 18.3 per cent (Fig. 2). The large increase in beta globulin to 28.2 per cent may be attributed in part to the high serum cholesterol (714 mg. per cent).

Cancer of the Liver

The protein fractions were analyzed electrophoretically in 7 cases of metastatic carcinoma of the liver (Fig. 5). The primary source of the carcinoma was the pancreas in 5 cases (Table 5, Cases 37, 54, 58, 60, 64), the stomach in 1 (Table 5, Case 57), and the rectum in 1 (Table 5, Case 45). The diagnosis was confirmed by autopsy or biopsy in 5 of the 7 patients. Jaundice was quite pronounced in each instance, the icteric index varying between 40 and 200.

The serum albumin-globulin ratio, determined by the fractional precipitation method, was normal in 2 of the 5 cases studied. Electrophoretically, however, the albumin was moderately decreased in 6 of the 7 cases, and varied between 39.5 and 60.6 per cent, with an average of 48.8 per cent.

Abnormalities of the serum globulins are less prominent in secondary carcinoma of the liver than in any other form of intrahepatic disease. The gamma globulin was essentially normal in 4 of the 7 cases, and was increased in the 3 remaining cases. The increase in beta globulin noted in all 7 cases may be explained, in part, at least, by the high blood cholesterol values which were present in the 5 cases with carcinoma of the head of the pancreas. The beta globulin was moderately elevated, however, in 1 case (Table 5, Case 45) in which the blood cholesterol was normal.

Common Duct Obstruction

Jaundice alone does not produce significant alteration of the serum proteins (Fig. 6). This fact was demonstrated in 5 patients with a common duct stone, whose livers appeared grossly normal on surgical exploration. The jaundice was quite severe in all cases, as illustrated by icteric indices of 30, 32, 68, 93, and 125. Although the serum albumin was decreased to 50.3 and 53.6 per cent in 2 cases, the gamma globulin was essentially normal in every case. It is interesting to observe that the beta globulin was normal in 2 cases, slightly elevated in 2 cases, and increased to 25.8 per cent in 1 case, although the serum cholesterol determinations were greatly increased in all 5 cases (250 to 416 mg. per cent) (Table 6).

Case No. 38 deserves special mention. There was in this patient an increase in the total protein concentration of the serum (9.25 per cent). The liver was enlarged; the icterus index was 68.1; the cholesterol content was 250 mg. per cent. Whether the large increase in B globulin (25.8 per cent) could be attributed to the increased cholesterol, as Longsworth seems to think, was not established. However, the patient when operated for cholelithiasis and cholecystitis (many gall stones were found in the gallbladder) showed in the liver focal areas of necrosis and pyknosis of the nuclei of liver cells. Judging by analogy with the other hepatitis, one is tempted to conclude that the increased beta globulin was a manifestation of the hepatic disorder, confirmed by the microscopic examination.

Albumin-Globulin Ratios Obtained by Electrophoretic Separation of Serum Proteins and by Fractional Precipitation

Electrophoretic analyses of the serum proteins yield lower albumin and higher globulin determinations, and consequently lower albumin-globulin ratios, than are obtained by fractional precipitation (Table 7), although occasionally the results may be identical by both methods.

It should be emphasized that the albumin-globulin ratio or the serum globulin may appear normal as determined by precipitation but definitely abnormal in the distribution of the globulin fractions by electrophoretic analysis. This is most often observed in the acute parenchymatous diseases of the liver, in which the serum proteins are usually normal when determined by precipi-

tation, but markedly abnormal on electrophoretic analysis. It is in these early acute stages of liver disease that these alterations of the globulin fractions are most frequently demonstrated electrophoretically, in spite of normal serum protein determinations on chemical analysis. This was clearly demonstrated in 3 cases of catarrhal jaundice (Table 4, Cases 32, 42, 68) and in 1 case of arsenical hepatitis (Table 4, Case 14), in which the serum globulin or the albumin-globulin ratio was normal by chemical analysis, while the globulin distribution was so abnormal electrophoretically that gamma globulin increases of more than 100 per cent were observed.

Similar but less frequent examples of abnormal globulin distribution with normal albumin-globulin ratios were seen in cirrhosis of the liver (Table 3, Case 20) and in metastatic carcinoma of the liver (Table 5, Case 58).

The differences in the albumin-globulin ratios obtained electrophoretically and by fractional precipitation are least discernible in cirrhosis of the liver. Here, the serum proteins are so definitely abnormal on chemical analysis that the albumin-globulin ratios determined by both methods approximate each other closely (Table 7). In metastatic carcinoma of the liver, however, the albumin-globulin ratios obtained electrophoretically are more abnormal than is suggested by the chemical determinations, while the A/G ratios in common duct stone are essentially the same by both methods of analysis, and are identical with the control studies.

COMMENT

These studies indicate that the diseased liver is unable to produce albumin as readily as the normal liver, and the more severe the hepatic insufficiency, the greater is the impairment of albumin production. Consequently, in cirrhosis of the liver with severe diffuse hepatic damage, the protein abnormalities are more pronounced than in any other form of liver disease (Table 3). Albumin values below 40 per cent were observed in 6 cases of cirrhosis of the liver in the late stages of hepatic insufficiency, demonstrating the relationship of severity of disease to impairment of albumin production. The effect of external protein loss will be discussed later. In general, the mean serum albumin was

lower in cirrhosis of the liver than in any of the other liver diseases.

Although the degree of liver damage and hepatic insufficiency was considerably less in the patients with acute parenchymatous hepatic involvement, marked impairment of albumin production was observed (Table 4). The mean serum albumin determination was 48.5 per cent and values as low as 40.3 and 42.8 per cent were noted, although many of these patients were seen in the early stages of the disease, and none had ascites or albuminuria.

Extensive carcinomatous involvement of the liver caused impaired albumin production, depending upon the degree of liver insufficiency. The lowest albumin value of 39.5 per cent was seen in a patient with extensive carcinomatosis of the liver, without ascites, observed on surgical exploratory examination (Table 5, Case 58). Values below 50 per cent were observed in 3 other cases, in 1 of which, ascites was present (Table 5, Case 57). The albumin determinations were not uniformly low in this group, however, as may be noted by such values as 52.7, 58.6, and 60.6 per cent in cases with less extensive liver involvement.

The serum albumin determinations were essentially normal in 3 of the 5 cases of extrahepatic jaundice, although some impairment of albumin production, to 50.3 and 53.6 per cent, was seen in 2 cases. Prolonged jaundice alone may cause early liver damage, and the decreased values may reflect these changes.

It would appear from these observations that the serum protein changes in liver disease result primarily from the inability of the liver to produce normal serum proteins, rather than to external loss of protein to the ascitic fluid. This is in agreement with the observations of several investigators. The albumin-globulin abnormalities may be as severe in the absence of ascites as in cases with massive ascites. This may be confirmed by comparing albumin values of 40.3, 42.8, 44.5, and 39.5, observed in patients with liver disease without ascites, to albumin values of 52.8, 58.8, 42.7, 39.9, and 39.6, noted in patients with ascites. In the 12 patients with cirrhosis of the liver, 6 had ascites and 6 did not. The mean albumin determination was 44.7 per cent in the former group, and 46.8 per cent in the latter, with as many albumin determinations below 40 per cent in one group as in the other. It must be concluded from these studies that although the protein abnormalities may be slightly more pronounced in the patients with ascites than without it, the abnormal protein

determinations result primarily from the liver insufficiency itself.

The more severe the impairment of albumin formation in the liver, the greater is the attempt by the body to compensate with an increased output of beta and gamma globulins, especially the latter. Consequently, in cirrhosis of the liver, where the albumin values are lowest, the gamma globulin determinations are highest, constituting 44.4 and 49.0 per cent of the total protein in some cases, and averaging 29.3 per cent, more than twice the normal gamma globulin value.

The most consistent and characteristic globulin abnormality in liver disease is the increase in the largest molecular weight fraction, the gamma globulin, and the impaired production of the smallest molecular weight protein, the albumin. These changes are seen most frequently, and to the greatest degree, in cirrhosis of the liver, and next most frequently, in the acute parenchymatous diseases in which the gamma globulin determinations of 44.2 and 30.9 per cent were observed, although the average for this group was 25.9 per cent. The least pronounced gamma globulin abnormalities occurred in cancer of the liver. The gamma globulin values were all normal in extrahepatic jaundice.

It is of considerable interest to note that a definite increase in gamma globulin is observed on electrophoretic analysis in many instances in which the serum globulin and albumin-globulin ratio appear to be normal as determined by fractional precipitation. That this occurs in the acute parenchymatous disease of the liver most frequently is particularly significant in that it suggests a possible mechanism for the positive serum colloidal gold reactions which occur with presumably normal serum proteins in patients with catarrhal jaundice and arsenical hepatitis.

The increased beta globulin values are difficult to interpret since the blood lipoids migrate with the beta globulin. It should be mentioned, however, that in 5 patients with common duct obstruction and high blood cholesterol determinations between 250 and 416 mg. per cent, the beta globulin was elevated appreciably in only 1 case and to a lesser degree in 2 others. Significant increases in beta globulin (i.e., a beta globulin increase in the presence of normal blood cholesterol) were observed in all the types of liver disease studied, but to a considerably less degree and frequency than the gamma globulin changes. Beta globulin

values of 18.4, 20.6, and 22.5 per cent were observed in cirrhosis of the liver, 20.5 and 25.9 per cent in the acute parenchymatous diseases, and 18.6 per cent in metastatic carcinoma of the liver. Disregarding high blood cholesterol determinations, elevated beta globulin values were observed in 5 of the 12 cases of cirrhosis of the liver, 3 of 6 cases of acute parenchymatous liver disease, and in all 7 cases of metastatic carcinoma of the liver. In the latter, the blood cholesterol values were exceedingly high, varying between 340 and 600 mg. per cent, since the malignancy of the pancreas caused complete long-standing biliary obstruction in 5 of the 7 cases.

It is interesting to observe that in several cases in which the serum albumin was very low (below 40 per cent), and the beta and gamma globulin did not sufficiently compensate for the decrease in albumin, the alpha globulin was increased to 9.8 per cent or more (Table 3, Cases 25, 35, 36, 47, 62), indicating that in extreme cases where the liver does not adequately compensate by producing an increase in the larger beta and gamma globulin fractions, it may then produce an increase of the smaller alpha globulin as well.

Abnormalities of the serum globulins are less prominent in metastatic carcinoma of the liver than in any other form of intrahepatic disease. This may be explained by the fact that in this disease there are areas of cancer tissue dispersed through and surrounded by normal liver tissue, and since the reserve of the liver is very large, the serum proteins are not appreciably altered until the very late stages of the disease.

Jaundice alone causes little change in the serum proteins, as demonstrated in the 5 patients with common duct stone. Although the icteric indices were quite high in these patients, the serum globulins were essentially normal.

CONCLUSIONS

1. The most characteristic alteration of the serum proteins in liver disease is a large increase in the gamma globulin and a decrease in serum albumin. These changes are seen most frequently and to the greatest degree in cirrhosis of the liver and next most frequently in the acute parenchymatous diseases.

2. The distribution of the serum globulin fractions may

be definitely abnormal electrophoretically in spite of a normal albumin-globulin ratio on chemical analysis. An increase in gamma globulin is observed consistently in the acute parenchymatous diseases of the liver in which the serum globulin and albumin-globulin ratio appear to be normal as determined by fractional precipitation.

3. An abnormality of two or more protein fractions was observed in every case of liver disease studied. The degree of abnormality depended on the severity of the disease.

4. Electrophoretic analyses of the serum proteins yield lower albumin and higher globulin determinations, and consequently lower albumin-globulin ratios, than are obtained by fractional precipitation.

5. Significant increases in beta globulin were observed in all types of liver disease, but to a considerably lesser degree and frequency than the gamma globulin changes.

6. Abnormalities of the serum proteins are less prominent in metastatic carcinoma of the liver than in any other form of liver disease.

7. The serum protein changes in liver disease result primarily from the inability of the liver to produce normal serum proteins, rather than from external loss in the ascitic fluid.

8. Jaundice alone does not produce significant serum protein changes.

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TABLE 1

PERCENTAGE COMPOSITION OF SERUM PROTEINS
IN NORMAL SERUM

Patient	Total Protein	A/G Ratio	Albumin	Alpha	Beta	Gamma
1	7.13	4.98/2.15	65.4	6.2	12.6	15.7
2	7.30	4.55/2.75	62.5	7.9	14.6	15.0
3	7.60	4.75/2.85	62.8	7.5	15.1	14.6
4	7.00	4.81/2.19	65.5	6.4	15.0	13.1
5	7.40	.....	64.0	7.1	15.2	13.7

TABLE 2

RATIO OF CONCENTRATION OF EACH GLOBULIN ALBUMIN
CONCENTRATION FOR NORMAL SERUM

α/Albumin	β/Albumin	γ/Albumin	Reference
0.13	0.26	0.17	Svensson
0.12	0.23	0.20	Longsworth
0.11	0.21	0.19	Luetscher
0.12	0.21	0.26	Gutman
0.08	0.19	0.43	Kekwick
0.11	0.23	0.22	Gray and Barrón

TABLE 3

PERCENTAGE COMPOSITION OF SERUM PROTEINS
IN CIRRHOSIS OF THE LIVER

Patient	A/G Ratio	Albumin	Alpha	Beta	Gamma	Diagnosis
7	3.60/3.04	52.8	8.6	22.5	16.1	Atrophic cirrhosis (autopsy)
15	4.77/3.22	45.4	4.8	30.7	19.1	Xanthomatosis and biliary cirrhosis (biopsy)
20	5.11/2.54	64.5	3.5	18.4	13.6	Atrophic cirrhosis
22	3.06/3.45	36.0	5.1	14.5	44.4	Atrophic periportal cirrhosis (autopsy)
25	1.98/2.90	38.1	10.5	20.6	30.8	Atrophic cirrhosis (peritoneoscopy)
28	3.12/2.29	58.8	9.1	13.6	18.5	Hypertrophic periportal cirrhosis (autopsy)
31	4.37/3.13	64.3	4.6	9.8	21.3	Pellagra and atrophic cirrhosis
35	2.94/4.41	42.7	10.1	12.0	34.2	Atrophic cirrhosis
36	.....	39.9	11.7	9.2	39.2	Polyserositis and capsular cirrhosis (autopsy)
47	2.63/3.92	39.6	9.8	14.4	36.2	Atrophic cirrhosis (autopsy)
61	3.23/6.02	35.1	4.5	11.4	49.0	Hemochromatosis and cirrhosis
62	.....	31.6	10.0	28.5	29.9	Hypertrophic cirrhosis (autopsy)

TABLE 4

PERCENTAGE COMPOSITION OF SERUM PROTEINS IN ACUTE
PARENCHYMATOUS DISEASES OF THE LIVER

Patient	A/G Ratio	Albumin	Alpha	Beta	Gamma	Diagnosis
14	5.62/2.23	44.5	9.0	28.2	18.3	Acute arsenical hepatitis (biopsy)
29	8.75/3.71	42.8	5.8	20.5	30.9	Catarrhal jaundice (biopsy)
32	4.33/2.45	40.3	7.5	8.0	44.2	Catarrhal jaundice
33	3.16/3.14	48.8	9.1	25.9	16.2	Catarrhal jaundice
42	3.79/2.75	56.7	7.7	13.4	22.2	Catarrhal jaundice
68	3.91/2.25	58.1	6.0	10.1	25.8	Catarrhal jaundice

TABLE 5

PERCENTAGE COMPOSITION OF SERUM PROTEINS IN METASTATIC CARCINOMA OF THE LIVER

Patient	A/G Ratio	Albumin	Alpha	Beta	Gamma	Primary Source of Liver Metastases
37	5.42/3.56	52.7	8.6	24.2	14.5	Pancreas (biopsy of liver)
45	3.59/3.99	45.3	6.9	18.6	28.2	Rectum (autopsy)
54	5.62/2.76	60.6	8.0	19.7	11.7	Pancreas
57	.....	40.0	8.6	29.2	22.2	Stomach (autopsy)
58	4.15/2.36	39.5	4.7	18.4	37.4	Pancreas (biopsy of liver)
60	.....	58.6	9.1	20.4	11.9	Pancreas
64	3.55/2.26	44.7	8.7	30.1	16.5	Pancreas (biopsy of liver)

TABLE 6

PERCENTAGE COMPOSITION OF SERUM PROTEINS IN COMMON DUCT STONE

Patient	Total Protein	A/G Ratio	Albumin	Alpha	Beta	Gamma
38	9.25	5.47/3.78	50.3	8.6	25.8	15.3
43	7.19	4.59/2.60	66.7	8.1	18.3	6.9
44	....	.....	53.6	9.1	19.6	17.6
63	6.90	3.91/2.99	60.7	7.6	14.8	15.9
71	6.86	4.24/2.62	64.6	7.6	14.6	13.2

TABLE 7

ALBUMIN-GLOBULIN RATIOS OBTAINED BY ELECTROPHORETIC SEPARATION OF SERUM PROTEINS AND BY FRACTIONAL PRECIPITATION

	$\frac{\text{Albumin}}{\text{Globulin}}$ Ratio	
	Electrophoresis	Fractional Precipitation
Normal	1.89	2.31
Normal	1.90	2.19
Normal	1.67	1.65
Normal	1.69	1.67
Acute hepatitis and cholangitis (biopsy)	0.75	1.01
Catarrhal jaundice	0.95	1.00
Catarrhal jaundice	0.67	1.76
Catarrhal jaundice	1.30	1.38
Catarrhal jaundice	1.38	1.73
Arsenical hepatitis (biopsy)	0.81	2.52
Cirrhosis of liver with ascites (biopsy)	0.65	0.67
Diabetes mellitus and cirrhosis of liver	0.54	0.54
Atrophic cirrhosis of liver (autopsy)	0.56	0.88
Xanthomatosis and biliary cirrhosis (biopsy)	0.83	1.48
Hypertrophic cirrhosis of liver (autopsy)	1.42	1.36
Carcinoma of pancreas with metastases to liver (biopsy)	1.12	1.52
Carcinoma of rectum with metastases to liver (autopsy)	0.86	0.90
Carcinoma of pancreas with metastases to liver (biopsy)	0.65	1.75
Carcinoma of pancreas with metastases to liver (biopsy)	0.81	1.57
Common duct stone	1.98	1.99
Common duct stone	1.72	2.43

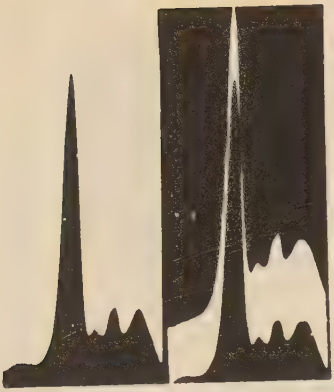
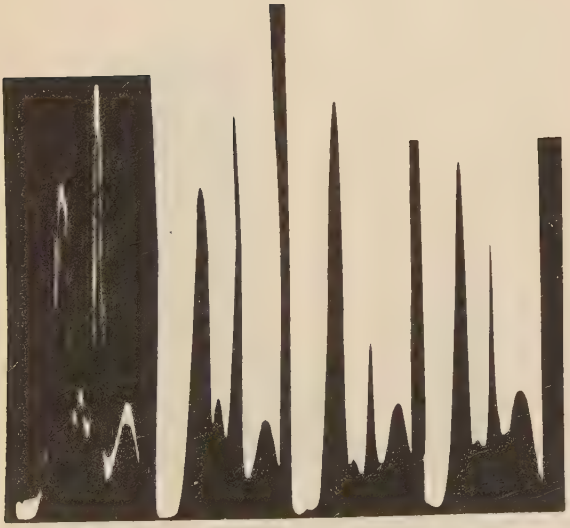
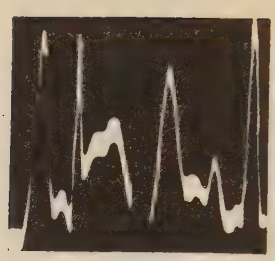


Fig. 1.--Normal serum



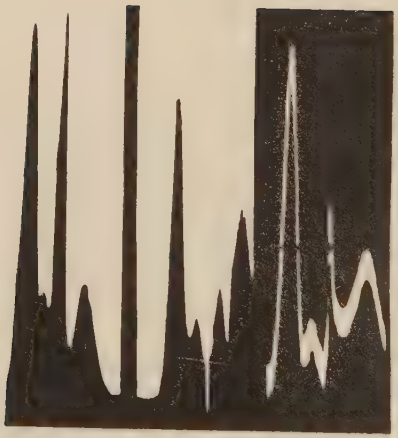
Arsenical hepatitis No. 14 | Arsenical hepatitis No. 14 | Catarrhal jaundice No. 42 | Acute hepatitis No. 29

Fig. 2.--Acute parenchymatous diseases of the liver.



Cathode Anode

Fig. 3.--Catarrhal jaundice

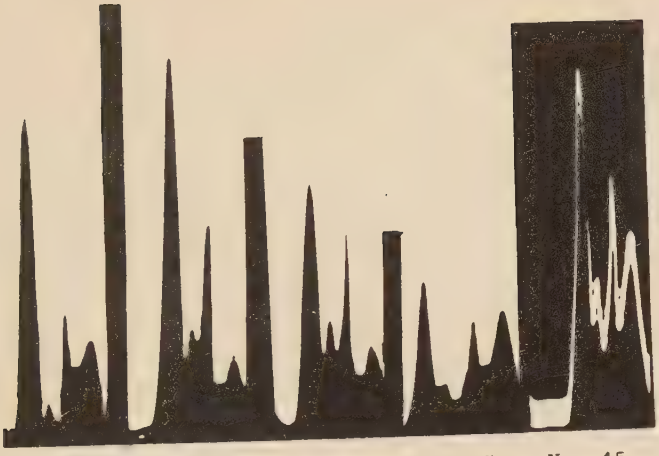


Xantho-matosis and cirrhosis No. 15

Cirrhosis of the liver No. 35

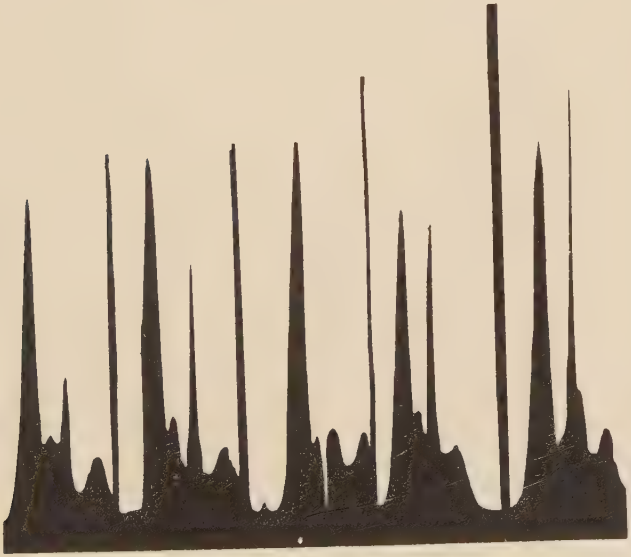
Cirrhosis and ascites No. 31

Fig. 4.--Cirrhosis of the liver



No. 54 No. 37 No. 60 No. 57 No. 45

Fig. 5.--Metastatic carcinoma of the liver



Common duct stone No. 71 | Common duct stone No. 44 | Common duct stone No. 63 | Common duct stone No. 43 | Common duct stone No. 38

Fig. 6.--Extrahepatic jaundice

PART III. THE MECHANISM OF THE COLLOIDAL GOLD REACTION

A. Mechanism of the Colloidal Gold Reaction of Blood Serum in Liver Disease

B. Mechanism of the Spinal Fluid Colloidal Gold Reaction

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Section B Published in part in Proceedings of the Society for Experimental Biology and Medicine, 51: 401, 1942

INTRODUCTION

The evidence presented in Part I suggested that the serum colloidal gold reaction in hepatic disease depended upon an alteration within the serum globulin rather than upon a quantitative increase in the total globulin content of the serum. The exact mechanism of the reaction had not been determined.

It was concluded in Part II moreover, that the serum proteins in liver disease were characterized by an increase in the gamma globulin fraction and a decrease in the serum albumin.

The flocculating action of the globulin fraction and the inhibitory effect of albumin upon colloidal gold suspensions has been established by many observers (1). In an effort to determine the mechanism of the colloidal gold reaction in hepatic disease, it was thought desirable to investigate the effect of electrophoretically pure protein fractions upon the serum colloidal gold reaction and upon the suspension stability of colloidal gold solutions in general.

It has been impossible, heretofore, to study the effect of the individual globulin fractions on colloidal gold precipitation because these fractions could not be obtained in pure form. Tiselius (2) has shown that the precipitation ranges of the globulin fractions overlap one another grossly and that the euglobulin, pseudoglobulin, and albumin fractions prepared by the usual methods of fractional precipitation (3) are mixtures of albumin, alpha, beta, and gamma globulins.

The electrophoretically pure protein fractions prepared by Dr. Edwin J. Cohn (4) presented an opportunity to study the effects upon colloidal gold flocculation of pure human albumin, gamma globulin, and the fraction containing alpha and beta globulins.

A. MECHANISM OF THE COLLOIDAL GOLD REACTION OF BLOOD SERUM IN LIVER DISEASE

METHOD OF STUDY

Electrophoretically pure protein fractions prepared from human serum by Dr. Edwin J. Cohn (4) were used throughout these studies. The albumin and the alpha and beta globulins were isoelectric while the gamma globulin was in the salt form. The electrophoretically pure human protein fractions were added to normal serums in increasing concentrations from 0.2 to 2.0 Gm. per cent. Albumin was added to the serum of a patient with cirrhosis of the liver as well as to normal serum. The resulting increase in each protein fraction was roughly equivalent to the increases observed on electrophoretic analysis of the serum proteins in the various types of liver disease studied in Part II.

The serum was then diluted 1:350 with 0.9 per cent sodium chloride, and the blood serum colloidal gold reaction was performed in the usual manner except that 3 dilutions were made instead of 10. Five cc. of properly acidified colloidal gold were added to each of 3 tubes containing 1 cc. of serum in final dilutions of 1:3500, 1:7000, and 1:14,000. The tubes were allowed to stand at room temperature, and were read after 24 hours in the usual manner described in Part I.

RESULTS

The addition of 0.2 Gm. per cent of electrophoretically pure human gamma globulin to normal blood serum produced a positive colloidal gold test--522--which increased in intensity as the concentration of the gamma globulin was increased to 1.0 Gm. per cent. At this point all 3 tubes showed complete flocculation--555 (Table 1).

Similar amounts of the purified fraction containing alpha and beta globulin were added to normal serum and produced no effect whatever upon the blood serum colloidal gold reaction (Table 1).

Pure albumin inhibited the serum colloidal gold reaction. The addition of 0.8 Gm. per cent of albumin to normal blood serum inhibited the flocculation from 222 to 000. When albumin was

added to strongly positive serum, complete flocculation of the colloidal gold was inhibited in the second tube by the addition of 0.4 Gm. per cent of albumin, and inhibition of the positive reaction in the first tube occurred when 2.0 Gm. per cent of albumin was added to the serum (Table 1).

Control studies with equivalent concentrations of sodium chloride did not cause flocculation in any dilution.

No significant alteration of pH was observed in the blood sera containing the purified protein fractions.

COMMENT

It is interesting to observe that the addition of only 0.2 Gm. per cent of gamma globulin to normal serum flocculates the colloidal gold suspension. This is equivalent to an increase of only 3 per cent of the gamma globulin of the serum. Since the gamma globulin content of the serum is increased by an average of 15 per cent in cirrhosis of the liver and 10 per cent in the acute parenchymatous disease of the liver (Part II) the extreme sensitivity of the reaction in these diseases is readily understood.

The inhibitory activity of albumin upon colloidal gold flocculation is another important factor in the reaction. The diseased liver is unable to produce albumin normally, and the more severe the liver disease is the greater is the impairment of albumin production. Albumin production in cirrhosis of the liver is reduced by an average of 16 to 20 per cent which is equivalent to approximately 1.4 Gm. per cent of albumin. In the acute parenchymatous diseases of the liver the average serum albumin content is reduced by 14 to 18 per cent or an equivalent of approximately 1.2 Gm. per cent of albumin (Part II). Thus the inhibitory effect of the albumin fraction upon colloidal gold flocculation is reduced in these diseases permitting even greater flocculation by the gamma globulin fraction of the serum.

In carcinoma of the liver the alteration of the serum albumin and gamma globulin is not as consistent as in cirrhosis of the liver and the acute parenchymatous diseases (Part II), and consequently the colloidal gold reaction is not as sensitive in this type of hepatic involvement.

Although a 15 per cent increase in beta globulin may occur in certain cases of cirrhosis of the liver or catarrhal jaundice

(Part II), this fraction, apparently, does not effect the flocculation of colloidal gold suspensions, since the addition of equivalent concentrations of this fraction to normal serum does not alter the reaction in any manner.

It should be emphasized that the flocculating effect of gamma globulin upon colloidal gold suspensions is not attributable to a change in the salt concentration or acidity of the solutions.

Since the most characteristic and consistent alteration of the serum proteins in hepatic disease is a decrease in serum albumin and a relatively large increase in gamma globulin (Part II), it is suggested that the mechanisms of the colloidal gold reaction in liver disease depends upon a relative increase in the gamma globulin of the serum.

It was observed in Part II that the impairment of serum albumin production and the increment in the gamma globulin are related to the degree of liver damage. The studies in this section indicate that the degree of flocculation increases with the relative increase in the gamma globulin content of the serum (Table 1). In this manner there is obtained an index of the degree of liver damage. Flocculation in the first dilution indicates mild liver damage, and in the second tube moderately severe hepatic involvement. Flocculation in all three dilutions indicates extensive and very severe liver damage, and the prognosis in these instances is very guarded. The 555 reaction has predicted correctly a fatal outcome in many cases of hepatic disease and may be equal in prognostic significance to other available methods used in the evaluation of the degree of liver involvement.

The actual physico chemical mechanism by which albumin inhibits and gamma globulin promotes flocculation of colloidal gold suspensions requires further discussion. The colloidal gold suspension is a hydrophobic sol which remains stable by virtue of the mutually repellent surface charge on the individual particles (5). These colloidal gold particles are negatively charged. As soon as their surface potential is depressed below a critical level, as by the addition of electrolytes, the individual gold particles no longer mutually repel each other but actually cohere to form unstable and sedimenting aggregates of increasing size. This is referred to as flocculation.

The color of the colloidal gold suspension and the degree of flocculation depend upon the particle size. The average particle size of the stable colloidal gold suspension is 20-25 milli-

microns. As the particle size increases the color changes from red to orchid to blue until complete flocculation results in a colorless supernatant fluid. At this point the particle or aggregate size approximates 35-40 millimicrons.

The electrolyte ionic strength and temperature remaining constant, the stability of the colloidal gold solution depends upon the hydrogen ion concentration and the particle size (6). In view of the fact that gold sols are negatively charged, an increase in the concentration of negative ions increases the negative charge upon the particles and increases the stability of the suspension. Thus the more alkaline the colloidal gold sol is the more stable is the suspension. The particle size is of importance also in that the smaller the particle size, the greater is the mutually repellent surface potential and the more stable is the solution.

It may be postulated that when a colloidal gold suspension at pH 7.2 is added to blood serum with a high gamma globulin content, as from a patient with liver disease, the gamma globulin is adsorbed by the individual particles of the colloidal gold suspension and forms a sensitizing film of gamma globulin around the colloidal gold particles. At pH 7.2 gamma globulin has the lowest electrophoretic mobility and the weakest charge of any of the protein fractions by virtue of its high isoelectric point. In view of the fact that the pH of the solution (7.2) approaches the isoelectric point of gamma globulin (6.4) the surface potential of the colloidal gold particles is depressed, whereupon the particles cohere and precipitate in the presence of electrolytes (5).

Albumin, on the other hand, has the lowest isoelectric point (4.6) and the greatest negative charge and electrophoretic mobility of any of the protein fractions at pH 7.2 of the colloidal gold suspension. Consequently when it is adsorbed by the colloidal gold particles it increases the mutually repellent surface potential and stabilizes the suspension. In this manner the absorbed surface film of albumin is said to function as a protective colloid and inhibits the flocculation of colloidal gold suspensions.

In view of the association of the colloidal gold reaction with the gamma globulin fraction the question arises as to the source of the blood proteins. All available evidence indicates that the liver is the primary site of protein synthesis and is associated intimately with the formation of plasma proteins (7).

The liver is not only the principle site of protein storage and synthesis but it also regulates the mechanism by which a "dynamic equilibrium" is maintained between the stored and circulating protein of the blood (7).

In regard to protein synthesis there is complete dissociation of the individual factors in the liver which contribute to the production of albumin, globulin, and fibrinogen (8). There is a similar dissociation in the production of the plasma proteins (9).

In experimental liver injury the plasma fibrinogen level falls rapidly and in proportion to the degree of liver injury (9b, 11), hepatectomized animals show a rapid decrease in blood fibrinogen content. There is general agreement that fibrinogen production depends entirely upon the liver (10).

The liver plays an important role in the production of plasma albumin (8, 10). The evidence presented in Parts I and II clearly indicates that hepatic disease is associated with a marked reduction in the albumin content. Kerr, Hurwitz, and Whipple noted a definite lag in the regeneration of the plasma proteins, particularly the albumin fraction, in Eck fistula dogs following acute plasma depletion (12). Additional studies with hepatectomized dogs and animals with experimental liver injury indicate an impairment of plasma protein production, particularly the albumin and fibrinogen fractions (10).

The origin of the globulin fractions has not been determined definitely. Whipple believes that a considerable portion of the globulin is formed by the liver (10). Cutting and Cutter observed, however, that a reticulo-endothelial blockade produced by india ink injections abolished the ability of the rat to replace acute loss of protein, particularly globulin (13). Sabin has suggested that gamma globulin is formed in the cells of the reticulo-endothelial system (14). It is not known, however, to what extent the reticulo-endothelial system in general effects the concentration of gamma globulin in the blood serum or what part of the extrahepatic reticulo-endothelial system is concerned with gamma globulin formation. The possibility that the reticulo-endothelial system of the liver alone may play an important role in the formation of gamma globulin must be considered.

Most observers have concluded that the alteration of the blood proteins in hepatic disease is not due to the loss of protein in the ascitic fluid, inadequate dietary intake, or lack of

protein absorption but to abnormal liver function (7, 15, 16).

It was noted in Part I that the colloidal gold reaction was positive in 8 of 20 cases of syphilis and in certain instances of subacute bacterial endocarditis, and passive congestion of the liver secondary to cardiac decompensation. Positive reactions were obtained, likewise, in rare instances of multiple myeloma, lymphopathia venereum, and lupus erythematosis.

The positive reactions in syphilis may be attributed to two factors. In the first place, hepatic involvement following antiluetic therapy is not an uncommon occurrence (17) as was discussed in Part I. Secondly, a decrease in the serum albumin and an increase in the gamma globulin fraction may occur in the sera of patients with lues (18, 19). However, these alterations in the protein fractions are by no means invariable. The electrophoretic patterns may be entirely normal in syphilis (18). Cooper observed recently that all fractions of the serum giving positive serological reactions contain beta and gamma globulins (19), although "an increase in all the globulin components was found with a decrease in the concentration of the albumin component" (19).

Positive reactions were observed very rarely in the acute infectious diseases studied in Part I. The colloidal gold reaction was negative in cases of scarlet fever, peritonitis, mastoiditis, erysipelas, rheumatic fever, tuberculosis, and other infectious processes in which the liver was found normal at autopsy (Part I). In the cases of subacute bacterial endocarditis in which the reaction was positive, fatty degeneration of the liver was found invariably at autopsy. Fatty degeneration of the liver was observed at post mortem examination in other infectious processes, like pneumonia, in which positive colloidal gold reactions were obtained.

Although the largest proportion of the immune bodies are contained within the gamma globulin component of the serum, the increase in the gamma globulin in infectious diseases is not reflected in the serum to a sufficient degree to affect the colloidal gold reaction. Longsworth (20), for example, observed an increase in the alpha component of the serum of patients with pneumonia. The beta and gamma fractions were normal, and the albumin was decreased.

The colloidal gold reaction was found positive in Part I only occasionally in multiple myeloma, lymphopathia venereum, and

lupus erythematosis. In this group of diseases the protein fractions are quite variable. Kabat and his associates demonstrated an increase in serum gamma globulin and a decrease in albumin in patients with lymphopathia venereum (18). In other instances of this disease the protein fractions were normal. These studies may explain the occasional positive reaction found in the serum of patients with this disease.

The protein fractions in multiple myeloma may be normal (20, 21, 22). In some instances the gamma globulin component is increased, but more often there is an increase in the beta globulin (20, 21, 22). Similarly, in lupus erythematosis the serum protein components may be normal or there may be an increase in the beta or gamma fractions (23).

The serum colloidal gold reaction was negative in the two cases of Boeck's sarcoid studied in Part I. Fisher and Davis (24) observed that the sera of one-third of their patients with this disease demonstrated almost normal patterns on electrophoretic analysis, there being only a slight decrease in albumin and increase in alpha globulin. An elevation of the gamma globulin at the expense of albumin was observed in the sera of the remaining patients.

The consistently negative colloidal gold reaction observed in Part I in cases of nephrosis and nephritis with increased serum globulin determinations are explained readily by the fact that in these diseases there is a consistent decrease in the gamma globulin component with an increase in the alpha and beta components at the expense of the albumin fraction (25).

It should be emphasized, finally, that "actually the five components of different electrophoretic mobilities may be reasonably regarded as chemical genera rather than species of molecules, and hence gamma globulin probably represents a large group of chemically similar but not identical molecules" (24). The gamma globulin involved in the colloidal gold reaction may be actually produced in the liver (26) and may be different from the gamma globulin found in immune sera, multiple myeloma, syphilis, and the other diseases under discussion although there is no definite proof of this at present (22b).

SUMMARY AND CONCLUSIONS

1. The addition of electrophoretically pure gamma globulin to normal blood serum produced a positive colloidal gold test identical with that obtained with sera of patients with liver disease. The degree of flocculation increased with the increment in gamma globulin concentration.

2. The addition of pure alpha and beta globulin to normal serum had no effect upon colloidal gold flocculation.

3. Pure albumin inhibited the serum colloidal gold reaction. The addition of albumin to normal serum inhibited completely the flocculation of the colloidal gold suspension. When added to the serum of a patient with cirrhosis of the liver pure albumin converted the positive test to negative.

4. In view of the fact that the most characteristic and consistent alteration of the serum proteins in hepatic disease is a decrease in serum albumin and a relatively large increase in gamma globulin, it is suggested that the mechanism of the colloidal gold reaction in liver disease depends upon a relative increase in the gamma globulin of the serum.

B. STUDIES ON THE MECHANISM OF THE SPINAL FLUID COLLOIDAL GOLD REACTION

INTRODUCTION

In view of the fact that the gamma globulin in the serum of patients with liver disease flocculates colloidal gold suspensions yielding a curve similar to the dementia paralytica type of curve obtained with spinal fluid, it was thought advisable to investigate the effect of electrophoretically pure proteins upon the spinal fluid colloidal gold reaction. This study was made to elucidate further the mechanism of colloidal gold flocculation and to compare the reaction in the blood serum and in the cerebrospinal fluid of the same patients.

METHOD OF STUDY

Increasing amounts of pure human albumin, gamma globulin, and the fraction containing alpha and beta globulins in concen-

trations varying from 0.005 to 0.20 Gm. per cent were added to normal spinal fluids. The pure albumin fraction was added to the strongly positive spinal fluids of patients with paresis in concentrations of 0.05 to 0.8 Gm. per cent. Electrophoretically pure fibrinogen was added to normal spinal fluids in concentrations of 0.007 to 0.500 Gm. per cent.

The colloidal gold test of the spinal fluid was performed in the usual manner by the addition of 2.5 cc. of colloidal gold solution to 0.5 cc. of spinal fluid in serial dilutions of 1:10, 1:20, 1:40 to 1:5120. The tubes were allowed to stand at room temperature and were read after 24 hours. The blood serum colloidal gold reaction was performed as previously described.

RESULTS

Effect of Purified Protein Fractions on Colloidal Gold Flocculation

The addition of pure gamma globulin to normal spinal fluid produced a typical paretic colloidal gold curve--5555432100. Complete flocculation in the first tube was noted when 0.020 Gm. per cent of gamma globulin was added to normal spinal fluid (Table 2). The degree of flocculation increased with the increased concentration of the gamma globulin. The addition of 0.20 Gm. per cent of gamma globulin caused complete flocculation in 7 of the 10 dilutions (Table 2).

The addition of pure human albumin and alpha and beta globulin to normal spinal fluid produced no effect upon the flocculation of the colloidal gold (Table 2).

When pure human albumin was added to the spinal fluid of a patient with general paresis, flocculation of the colloidal gold was inhibited, and the paretic curve was converted to normal (Table 2). A strongly positive paretic colloidal gold curve was made to appear normal by the addition of 0.8 Gm. per cent of albumin. The inhibition of the colloidal gold flocculation was progressive as the concentration of albumin was increased from 0.05 to 0.8 Gm. per cent.

Fibrinogen appeared to produce a different type of flocculation--the mid-zone flocculation or the luetic type of curve (Table 3). Flocculation in the mid-zone began when 0.007 Gm. per cent of fibrinogen was added to normal spinal fluid and was com-

plete upon the addition of 0.500 Gm. per cent of fibrinogen (Table 3).

Comparison of the Colloidal Gold Reaction in the Blood Serum and in the Cerebrospinal Fluid

The colloidal gold reaction of the spinal fluid and that of the blood serum from the same patient may be the same or may differ greatly, depending on the disease process (Table 4). The colloidal gold reaction was positive in the blood serum and negative in the spinal fluid of 7 patients with liver disease. This group included 3 patients with syphilitic heart disease and large, hard livers (Table 4, Cases 10, 11, 12), 1 patient with portal cirrhosis (Case 25), 2 with arsenical hepatitis (Cases 13 and 14), and 1 with a hepatic tumor (Case 24).

In 4 cases of cerebrospinal syphilis, the colloidal gold reactions were positive in the spinal fluid and negative in the blood serum (Cases 1, 5, 7, 8). On the other hand, in 3 similar cases the colloidal gold reactions in both the spinal fluid and the blood serum were positive (Table 4, Cases 2, 3, 4). In 1 case (Case 9), in which both syphilis of the central nervous system and hepatic cirrhosis were present, both the spinal fluid and the blood serum gave positive reactions. In 3 cases of early syphilis with no evidence of hepatic disease (Cases 16, 17, 18) and in 3 cases of various conditions other than hepatic disease or syphilis (Cases 21, 22, 23) there were negative colloidal gold reactions in both the spinal fluid and the blood serum. In 1 case (Case 19) of meningococcic meningitis, with no clinically detectable hepatic disease, a positive colloidal gold reaction was obtained in the spinal fluid and a negative reaction in the blood serum.

COMMENT

It is apparent from these studies that gamma globulin produces the same type of colloidal gold flocculation with spinal fluid as it does with serum in that the typical paretic or first zone curve is produced in both. Kabat and his associates (18), reporting simultaneously upon this subject indicate that a considerable increase in gamma globulin occurs in the cerebrospinal fluid of patients with neurosyphilis. Kabat noted an increase in gamma globulin of 0.040 Gm. per cent in neurosyphilis, which agrees

well with the concentration necessary to yield a strongly positive paretic curve (Table 2), and concluded that gamma globulin produced the paretic curve.

In view of this evidence it appears very likely that the paretic curve obtained in the spinal fluid of patients with neurosyphilis is caused by an increase in the gamma globulin content of the spinal fluid.

The inhibitory effect of albumin on the spinal fluid colloidal gold reaction parallels the studies described for serum in this section. The type of curve obtained with spinal fluid in different diseases of the central nervous system must depend to a great extent upon the relative concentrations of the albumin and gamma globulin in the cerebrospinal fluid.

No conclusion can be reached concerning fibrinogen in the spinal fluid. Fibrinogen is frequently absent from normal spinal fluid but may be found in increased concentrations in certain inflammatory conditions of the central nervous system (18a). Exudative processes may certainly result in an output of fibrinogen in the spinal fluid. In such instances this protein fraction may be responsible for the mid-zone curve.

In neurosyphilis, however, just the contrary occurs. The reaction is positive in the spinal fluid and negative in the blood (Table 4). Kabat and his associates (18a) found that in neurosyphilis the gamma globulin is much higher in the cerebrospinal fluid than in the serum and concluded that some formation of gamma globulin takes place within the tissues of the central nervous system which is poured into the cerebrospinal fluid. These observations readily explain the positive colloidal gold reactions in the spinal fluid and the negative reactions in the blood serum of patients with neurosyphilis.

Alteration in the composition of the protein components of the serum may be reflected in the cerebrospinal fluid (18a). In the one case in which both syphilis of the central nervous system and hepatic cirrhosis were present the spinal fluid and serum both gave strongly positive colloidal gold reactions (Table 4). This could result from independent increases in gamma globulin in the serum and spinal fluid or from the transudation of the gamma globulin from the serum to the cerebrospinal fluid.

It is of interest that early syphilis does not alter the gamma globulin concentration of the serum or spinal fluid suf-

ficiently to effect the colloidal gold reaction. The reaction was negative in both the spinal fluid and the serum (Table 4). No specific correlation was observed between strongly positive Wassermann reactions in the serum and spinal fluid and positive colloidal gold reactions, although the spinal fluid Wassermann reaction was strongly positive in most instances in which positive spinal fluid colloidal gold reactions were observed.

CONCLUSIONS

1. The addition of pure gamma globulin to normal spinal fluid produced a typically paretic colloidal gold curve similar to that obtained in the serum of patients with hepatic disease.

2. Albumin inhibited colloidal gold flocculation. The addition of pure human albumin to the spinal fluid of patients with paresis converted the paretic curve to normal.

3. The addition of pure fibrinogen to normal spinal fluid produced a mid-zone type of colloidal gold curve.

4. Alpha and beta globulins did not affect the colloidal gold reaction.

5. The colloidal gold reaction of the spinal fluid and the serum in the same patient were identical or divergent depending upon the disease process and the permeability of the blood brain barrier. The relative concentrations of gamma globulin in the serum and spinal fluid were the deciding factors.

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TABLE 1

EFFECT OF PURE PROTEIN FRACTIONS UPON THE BLOOD SERUM COLLOIDAL GOLD REACTION

Amount of Protein Added Gm. Per Cent	Blood Serum Colloidal Gold Reaction		
	Gamma Globulin	Alpha and Beta Globulin	Albumin
0	222	222	222 554
.2	522	222	211 553
.4	532	222	111 532
.6	553	222	111 532
.8	553	222	000 532
1.0	555	222	000 522
2.0	555	222	000 322

TABLE 2

EFFECT OF PURIFIED PROTEIN FRACTIONS ON THE COLLOIDAL GOLD CURVE OF NORMAL AND PARETIC SPINAL FLUID

Amount of Protein Added Gm. Per Cent	Normal Spinal Fluid			Paretic Spinal Fluid. Albumin
	Globulin	and Globulins	Albumin	
0	0000000000	0000000000	0000000000	5553210000
.005	2211000000	0000000000	0000000000	.....
.01	2221110000	0000000000	0000000000	.....
.02	5221100000	0000000000	0000000000	.....
.03	5522110000	0000000000	0000000000	.....
.04	5553211000	0000000000	0000000000	.....
.05	5555432100	0000000000	0000000000	5543210000
.10	5555554210	0000000000	0000000000	5321000000
.20	5555555433	0000000000	0000000000	4211000000
.60	.....	.....	.....	1110000000
.80	.....	.....	.....	0000000000

TABLE 3

EFFECT OF PURE FIBRINOGEN ON THE COLLOIDAL GOLD CURVE OF NORMAL SPINAL FLUID

Amount of Protein Added Gm. Per Cent	Spinal Fluid Colloidal Gold Reaction
0	0000000000
0.007	0011000000
.015	0011110000
.022	0011210000
.030	0011220000
.037	0011221000
.045	0012211000
.060	0012211000
.090	0123111100
.120	0122320000
.200	0142211000
.500	0445532100

TABLE 4

COMPARISON OF COLLOIDAL GOLD REACTION OF BLOOD SERUM AND OF SPINAL FLUID

Case No.	Colloidal Gold Reaction		Wassermann Reaction		Globulin in Cerebrospinal Fluid	Diagnosis
	Blood Serum	Cerebrospinal Fluid	Blood	Spinal Fluid		
1	1221100000	4443331000	3+	4+	++	Cerebrospinal syphilis
2	5442110000	5555321000	4+	4+	++	Cerebrospinal syphilis
3	5555321000	5555321000	4+	4+	+	Cerebrospinal syphilis
4	4553210000	4553200000	4+	Neg.	+	Cerebrospinal syphilis
5	2321000000	5555321000	4+	4+	....	Cerebrospinal syphilis
6	1330000000	2223331110	4+	4+	++	Cerebrospinal syphilis
7	2221000000	5531000000	4+	Neg.	Neg.	Cerebrospinal syphilis
8	1210000000	5554321000	4+	4+	....	Cerebrospinal syphilis
9	5555310000	5555432100	4+	4+	++	Cerebrospinal syphilis and cirrhosis of liver
10	2532110000	0011000000	4+	Neg.	....	Syphilitic heart disease; large,hard smooth liver
11	1253110000	0000110000	3+	Neg.	Neg.	Syphilitic heart disease; large,hard liver
12	5521110000	1112000000	4+	4+	....	Syphilitic heart disease; large,hard liver
13	5552100000	0012000000	3+	Neg.	+	Secondary syphilis; history of toxic hepatitis
14	5532100000	0000100000	2+	Neg.	Neg.	Primary syphilis and arsenical hepatitis
15	1221000000	0000000000	4+	Neg.	Neg.	Primary syphilis
16	0220000000	0000000000	4+	Neg.	Neg.	Primary syphilis
17	2210000000	0010000000	3+	....	....	Primary syphilis
18	0232110000	0000110000	4+	....	....	Primary syphilis
19	0122100000	4441113321	Neg.	Neg.	+++	Meningococcic meningitis
20	0122100000	0000210000	Neg.	Neg.	Neg.	Biliary tract neoplasm; liver normal (autopsy)
21	2221000000	0011000000	Neg.	Neg.	....	Parkinsonism
22	0110000000	0001210000	Neg.	Neg.	+	Miliary tuberculosis; liver normal (autopsy)
23	1232100000	0011000000	Neg.	Neg.	+	Epidural abscess; liver normal (autopsy)
24	0454321000	0010000000	Neg.	Neg.	....	Primary tumor of liver (autopsy)
25	5544531000	0012100000	Neg.	Neg.	....	Portal cirrhosis of liver

PART IV. THE CLINICAL APPLICATION OF THE SERUM COLLOIDAL GOLD REACTION

A. Hepatolenticular Degeneration

B. Diseases of the Gallbladder

C. Diabetes Mellitus

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Section B Published in the Archives of Internal Medicine, 72: 176, 1943

INTRODUCTION

It was concluded from the studies in Part I that the blood serum colloidal gold reaction is a sensitive method of detecting liver disease. It was thought desirable, therefore, to investigate further the clinical application of this method to the study of (A) hepatolenticular degeneration, (B) diseases of the gallbladder, and (C) diabetes mellitus.

A. LIVER FUNCTION STUDIES IN HEPATO-LENTICULAR DEGENERATION

INTRODUCTION

The application of the serum colloidal gold reaction and other tests of liver function is of particular interest in hepatolenticular degeneration in that the usual tests of hepatic function have failed to demonstrate liver insufficiency in spite of the extensive cirrhosis of the liver which is a constant feature of the disease and is always found at autopsy (1, 2, 3).

REVIEW OF LITERATURE

When Wilson (4) described his series of cases of progressive lenticular degeneration in 1912 he noted: "The most curious and the most remarkable feature of this familial disease is the constant presence of a profound degree of cirrhosis of the liver. This hepatic cirrhosis does not reveal itself by any symptoms during life; nevertheless it is always found after death." Since then all investigators (1, 2, 3, 4) have agreed that extensive cirrhosis of the liver is always found at autopsy in the syndrome of hepatolenticular degeneration bearing Wilson's name and that this severe hepatic involvement is a constant, essential and cardinal characteristic of the disease without which the diagnosis cannot be made.

Wilson and Bruce (1) and others (3, 5) expressed the be-

lief, moreover, that hepatic cirrhosis may be the primary disease which actually precedes the lenticular lesions. Reports by Barnes and Hurst (2) and Jendralski (5b) indicate that hepatitis may kill a patient before lenticular signs or lesions appear and that this hepatic disease may occupy the foreground before there is any suspicion of a pathologic condition of the central nervous system. It is agreed generally that extensive hepatic disease is present before neurologic symptoms manifest themselves.

In spite of the prominent role attributed to the liver in hepatolenticular degeneration, symptomatic evidence of hepatic disease is slight or entirely absent. Occasionally there is a history of jaundice prior to the onset of nervous symptoms, and Jendralski (5b) reported 1 case in which a Kayser-Fleischer ring developed, and death from hepatic cirrhosis and ascites occurred before any nervous symptoms developed. Barnes and Hurst (2) and others (1, 3, 4) have emphasized the paucity of clinical symptoms contrasted with the severity of the cirrhosis and stated that no conclusive clinical evidence of hepatic disease has been obtained in most cases until a post mortem examination was made. The usual tests of hepatic function are particularly disappointing. Lüthy (3), Wilson and Bruce (1), and Barnes and Hurst (2) reported that the various tests of hepatic function they had performed were entirely inconclusive in demonstrating hepatic insufficiency in this disease.

The present section is devoted to a study of liver function in hepatolenticular degeneration utilizing the serum colloidal gold reaction and other sensitive tests of hepatic function.

METHOD OF STUDY

Nine patients with hepatolenticular degeneration were selected for this study. The chemical investigations include determinations of the serum bilirubin, albumin, globulin, cholesterol, and cholesterol esters. The following liver function tests were performed: galactose tolerance, bromsulphalein retention, five-hour dextrose tolerance, hippuric acid excretion, bilirubin excretion, prothrombin time, and colloidal gold reaction of the serum. The latter test was performed as described in Part I except that 3 serial dilutions were made instead of 10. Complete flocculation of the colloidal gold in one or more tubes (532)

was considered a positive test.

RESULTS

Serum Bilirubin

No evidence of clinical or subclinical jaundice was found in any of the patients with hepatolenticular degeneration. The serum bilirubin was normal in every case studied except 1 (Table 1, Case 1), in which a slight increase of the serum bilirubin above the normal of 1 mg. per cent was found. The van den Bergh reaction was normal throughout.

Serum Proteins

An increase in serum globulin with a decrease in serum albumin was associated not infrequently with chronic hepatic disease, particularly with cirrhosis of the liver (6) as was noted in Parts I and II of this thesis. The serum proteins in the present studies, however, were normal in all but 2 cases (Table 1, Cases 3 and 7), in which the serum globulin was elevated above 2.8 Gm. per cent, the upper limit of normal, and the albumin diminished below the normal level of 4 Gm. per cent.

Cholesterol and Cholesterol Esters

Thannhauser and Shaber (7) first called attention to the low ratio of the blood cholesterol esters to total cholesterol in hepatic disease. Normally the cholesterol esters constitute at least 40 per cent of the total cholesterol. Jezler and others (8) have confirmed the abnormally low ratio of cholesterol to cholesterol esters in many cases of portal cirrhosis. The evidence presented in Part I indicates that this low ratio is more frequent in the acute parenchymatous disease than in chronic cirrhosis of the liver. An abnormal ratio was found in only 2 of the 7 cases of hepatolenticular degeneration studied (Table 1, Cases 4 and 6). In the remaining 5 the ratio was within the lower limits of normal.

Galactose Tolerance

Practically all reports have indicated that the galactose tolerance test (9) is a useless index of hepatic function in the absence of jaundice (10). The results obtained are in accord with

their concept. The galactose tolerance test gave normal results in the 5 cases in which it was performed.

Bromsulphalein Retention

Although the bromsulphalein test (11) has been reported by some investigators (12) to yield a high percentage of positive results in cases in which there is clinical evidence of early hepatic cirrhosis, the results with this test were disappointing. Only 2 patients retained 10 per cent or more of the dye thirty minutes after an intravenous injection of 5 mg. per kilogram of body weight (Table 1, Cases 3 and 7). One of these 2 had shown a normal retention of dye five years previously, although he had demonstrated definite nervous symptoms at that time (Table 2, Case 3).

Dextrose Tolerance

Von Fejér and Hetényi (13) first reported a diabetic-like type of dextrose tolerance curve with hepatic damage. This work has been confirmed for cirrhosis of the liver by Coller and Troost (14), Jacobi (15), and Coller and Jackson (16). They concluded that hepatic disease produces an abnormal dextrose tolerance curve characterized by a normal or low level of the blood sugar during fasting, a high level of blood sugar during the first or second hour, and hypoglycemia during the fourth and fifth hours, the so-called inverted V-type of curve.

Definitely abnormal dextrose tolerance curves were found in 4 of the 6 patients (Table 2, Cases 1, 2, 3, and 4). The level of capillary blood sugar during fasting was normal in every case, but in 4 of the 6 cases there was subsequent hyperglycemia, the sugar content becoming more than 200 mg. per cent within two hours, with a return to a normal level within three hours in all but 2 cases (Table 2, Cases 3 and 4), in which the hyperglycemia persisted. No definite hypoglycemic phase was noted except in 2 cases (Table 2, Cases 2 and 5), in which the level of capillary blood sugar fell below 60 mg. per cent. Two patients in this group did not show the characteristic hyperglycemic phase (Table 2, Cases 5 and 8), but the dextrose tolerance curves were not entirely normal, because of mild hypoglycemia in one and a somewhat delayed return of the level of capillary blood sugar to normal in

the other. The many factors entering into the metabolism of dextrose, including long-standing malnutrition and chronic loss of weight, make this curve a difficult one to interpret. The finding by Robinson and Shelton (17) of many diabetic-like curves for patients with nervous and mental diseases makes this series particularly difficult to evaluate.

Hippuric Acid Excretion

The hippuric acid excretion test introduced by Quick (18) in 1933 was considered a reliable test for hepatic cirrhosis (19). Excretion of less than 3 Gm. of benzoic acid in the urine within four hours of the oral administration of 5.9 Gm. of sodium benzoate to patients with normal renal function indicates hepatic disease. This test was positive in 3 of the 7 cases studied (Table 2, Cases 1, 4, and 8). One patient (Table 2, Case 7) vomited the sodium benzoate. The intravenous test (20) was resorted to, and the result was normal. Urinary excretion of more than 0.84 Gm. of benzoic acid in one hour was considered normal with this method.

Bilirubin Excretion

Von Bergmann and Eilbott (21) developed the bilirubin excretion test which Harrop and Barrón (22) and others (23) have described as the most delicate single test for impaired hepatic function. Retention of more than 6 per cent of the intravenously injected bilirubin at the end of four hours is considered abnormal. This test was found to be much more sensitive than any of the previous ones. It gave positive results in 5 of the 8 cases of hepatolenticular degeneration. One patient (Table 2, Case 2) had a normal excretion of bilirubin when the test was first performed, but after seven months of rapid progression of neurologic symptoms the test gave positive results.

Prothrombin Time

Determination of the prothrombin time has been found by S. J. Wilson (24) to be a much more sensitive indication of hepatic damage than either the hippuric acid or bromsulphalein tests and was noted by Allen and Julian (25) to persist as abnormal in 14 patients with advanced hepatic cirrhosis despite the administration of large doses of a vitamin K-like substance,

menadione, indicating that in the presence of a damaged liver prothrombin was not synthesized normally from vitamin K. In 6 of the 8 cases the prothrombin time was abnormal, a higher positive percentage than was obtained with any of the previous methods. Three patients (Table 2, Cases 1, 3, and 4) received 2 mg. of menadione four times daily for two to six days without exhibiting any change in the prothrombin time, thus providing further evidence that the abnormal prothrombin time was not due to inadequate intake of vitamin K but to the damaged liver. A large series of normal persons and patients with miscellaneous disorders not remotely involving the liver have shown a prothrombin time of 100 per cent.

Serum Colloidal Gold Reaction

Studies in Part I indicated that this test was particularly sensitive in cirrhosis of the liver giving positive results in a higher percentage of cases of clinical cirrhosis of the liver than any other test heretofore reported. Many of these cases of hepatic cirrhosis had been confirmed by autopsy or biopsy

It was concluded, likewise, in Part I that false positive reactions in normal individuals and patients with disorders unassociated with hepatic disease did not exceed 3 per cent. The control group, previously studied, included patients with numerous miscellaneous neurologic disorders, among which were 6 with paralysis agitans and 4 with parkinsonism following a definite attack of acute epidemic encephalitis. In these disorders the lesions involved the basal ganglions, presumably the same structures affected in hepatolenticular degeneration. In only 1 of the control cases was there an abnormal result, and repetition of this test did not confirm the first positive reaction.

The consistently positive results of the serum colloidal gold reaction in the first few cases of hepatolenticular degeneration under investigation instigated this study. The test gave positive results on 2 or more occasions in 8 of the 9 cases. In the 1 case in which the reaction was negative (Table 2, Case 8), hepatic disease was demonstrated by other methods.

Tables 1 and 2 summarize the determinations of the blood chemistry and the studies of hepatic function for the 9 cases. Evidence of disease of the liver was found in 8 cases by not less than three different methods, including the presumptive evidence

of the abnormal dextrose tolerance test. In 1 case (Tables 1 and 2, Case 1) six determinations were abnormal: the plasma bilirubin, five-hour dextrose tolerance, hippuric acid excretion, bilirubin excretion, prothrombin time, and serum colloidal gold reaction. In another case (Table 2, Case 5), however, only the bilirubin excretion and the serum colloidal gold reaction were abnormal.

COMMENT

Patients with hepatolenticular degeneration form excellent material on which to ascertain the delicacy of tests of hepatic function, since the clinical features of the lenticular degeneration in typical cases, in particular those in which there is a tremor which becomes grossly exaggerated on voluntary movement, are sufficiently distinctive to permit a diagnosis of the whole syndrome even in the absence of clinical evidence of disease of the liver (1, 26). The characteristic neurologic features of this disease, which is often familial and usually manifests itself before the age of 30, are tremor becoming definitely worse with voluntary movement or excitement; muscular hypertonia; masked facies or a fixed smile; slowed movement; occasionally tonic, clonic, or torsion spasms; dysarthria; dysphagia, and at times psychic changes.

Some of the cases show a further characteristic feature, the so-called Kayser-Fleischer ring, a deposition of yellowish-brown or greenish-brown pigment in Descemet's membrane near the limbus of one or both corneas, which in the presence of signs of extrapyramidal disease is pathognomonic of hepatolenticular degeneration (27). In the first 4 cases in the report the Kayser-Fleischer ring was seen on inspection with the naked eye, and the localization to Descemet's membrane was confirmed by study with a slit lamp and corneal microscope. No such pigment was seen in the other 5 cases.

It might be contended that with any patient presenting only neurologic symptoms there is no way of knowing that hepatic disease is already present. The following evidence favors the conclusion now generally held that the hepatic cirrhosis always occurs first and that the lenticular lesions follow. In the first place, in numerous cases cirrhosis of the liver has been demonstrated either at operation or on physical examination and only

subsequently have nervous symptoms developed (5d). Secondly, in several families in which one or more members presented the fully developed picture of hepatolenticular degeneration, others had died early in life from hepatic cirrhosis before neurologic symptoms appeared (2, 5). In other cases, moreover, there was a definite history of jaundice prior to the onset of any nervous symptoms (28).

In spite of the extensive cirrhosis of the liver which precedes the neurologic symptoms and is always found at autopsy, tests of hepatic function and other studies of hepatic insufficiency have failed, heretofore, to reveal conclusive evidence of pathologic conditions of the liver in hepatolenticular degeneration (1, 2, 3).

It is of considerable interest to observe that although previous studies of hepatic function in this disease have been disappointing and inconclusive (1, 2, 3) the serum colloidal gold reaction demonstrated liver disease in 8 of the 9 cases studied. None of the patients presented symptoms of hepatic insufficiency, and a normal-sized liver and spleen were found on physical examination in all cases.

CONCLUSIONS

1. In 9 cases of hepatolenticular degeneration, illustrating the features of this not infrequent disease, conclusive evidence of hepatic disease was demonstrated by tests of hepatic function for the first time.

2. The serum colloidal gold reaction appeared to be the most sensitive test for cirrhosis of the liver, demonstrating hepatic disease in 8 of the 9 cases studied.

3. The bilirubin excretion test and the prothrombin time were found to be very reliable indicators of hepatic disease.

4. Studies of the serum proteins, cholesterol, and cholesterol ester partition as well as the galactose tolerance and bromsulphalein retention were of little or no value in detecting hepatic disease. The hippuric acid test gave positive results in less than half of the cases studied.

B. INVOLVEMENT OF THE LIVER IN GALLBLADDER DISEASE

INTRODUCTION

Although the clinical demonstration of hepatic damage in cholecystic disease is of great importance in the medical and surgical management of disease of the biliary tract, there is little definite knowledge of this subject recorded in the literature. Studies of the incidence of hepatic involvement in disease of the gallbladder have not been conclusive and have not clarified the relationship between the two diseases. The purpose of this paper is to present new evidence of hepatic injury in disease of the gallbladder as detected by the serum colloidal gold reaction.

REVIEW OF LITERATURE

The presence of hepatic damage in disease of the gallbladder has been explained in three ways. One group of investigators (29) have stated the belief that the gallbladder is the primary source of infection and that the liver is involved by direct extension. Others have postulated that the liver acts as a bacterial filter of the body and is the chief source of the infection, involving the gallbladder secondarily by way of the bile ducts and lymphatics (30). A third group of workers (31) have maintained that the pathologic changes in the liver and those in the gallbladder are completely independent of each other.

After Riedel (32) in 1888 first demonstrated enlargement of the right lobe of the liver in cholecystitis, no critical work appeared until 1918, when Graham (33) described the pathologic involvement of the liver in 30 patients with disease of the biliary tract. Biopsy specimens taken at operation showed microscopic evidence of inflammation of the liver in 87 per cent of the patients with acute and subacute cholecystitis. MacCarty and Jackson (34) studied the liver histologically in 58 patients with acute, subacute, and chronic cholecystitis and demonstrated hepatic disease in 81 per cent. They could find no apparent relationship between the severity of the hepatic disease and the degree of inflammation of the gallbladder.

Judd in 1921 observed hepatitis at autopsy with infections of the gallbladder and biliary tract but never with peptic ulcer

or appendicitis (35). In 1924, however, Heyd, MacNeal, and Killian (36), investigating the incidence of hepatic disease in association with appendicitis and cholecystitis, found it as frequently with the former as with the latter. The degree of hepatic involvement was proportional to the chronicity of the infection. Mentzer (37) in 1926 made detailed post mortem studies of 548 patients with disease of the gallbladder. Sections for microscopic study were taken from the liver adjacent to the gallbladder and from the dome of the right and of the left lobe of the liver. The liver almost always showed hepatitis adjacent to the gallbladder, while sections taken from the left lobe were essentially normal even in patients with severe cholecystitis and cholelithiasis. Mentzer found that in 60 to 70 per cent of his patients there was pathologic evidence of hepatitis at post mortem examination regardless of the presence or absence of cholecystic disease. Noninflammatory lesions, including cholesterosis of the gallbladder, were associated with hepatitis in 97 per cent.

Noble (31b) studied the livers and gallbladders microscopically in 212 unselected autopsies. All but 5 of the subjects showed inflammatory cellular infiltration of the liver, although a large number had no evidence of disease of the gallbladder and none had clinical evidence of cholecystitis. Colp, Doubilet, and Gerber (38) made an intensive study of the liver in patients with acute and chronic cholecystitis, using special stains and taking specimens for biopsy deep in the parenchyma of the right and left lobes of the liver. They concluded that the periportal cellular infiltration observed in disease of the biliary tract is not specific and that there is no evidence that hepatitis is associated with cholecystic disease in the absence of jaundice. They observed focal cellular degeneration in the presence of jaundice and attributed these changes to bile stasis rather than to a primary disease of the gallbladder.

Although the majority of writers feel that there is a direct relationship between cholecystitis and hepatitis and that long-standing cholecystic disease leads to progressive damage to the liver, the evidence cited indicates that there are conflicting opinions as to the significance of mild cellular changes in the liver and that microscopic studies alone may not demonstrate conclusively significant disease and abnormal function of the liver.

Because of the liver's multiplicity of function and large

margin of reserve, the ideal laboratory test for its functional capacity is difficult to achieve. Tests of hepatic function in the past have not been sensitive enough to detect early involvement of the liver in cholecystic disease or have been too complicated to permit collection of a large series of cases. The bromsulphalein test, for example, cannot be evaluated in the presence of jaundice, and consequently its value in the study of disease of the gallbladder is limited. Of Cantarow's (39) series of 49 patients with acute cholecystitis, 41 had a normal serum bilirubin content and only 5, or 12.2 per cent, of these had an abnormal bromsulphalein retention. A similarly low incidence of hepatic involvement was found in 244 patients with chronic cholecystitis without gallstones and in 88 patients with calculous cholecystitis whose serum bilirubin determinations were normal. Abnormal bromsulphalein retention occurred in 32, or 13 per cent, of the first group and in 11, or 12.5 per cent, of the second.

Quick (18), in his original application of the hippuric acid excretion test to human beings, performed the test on 6 patients with disease of the biliary tract and found the liver normal in 4. Kohlsteadt and Helmer (40) found an abnormally low excretion of hippuric acid in 11 of 21 patients. The patients with normal excretion of hippuric acid made an uneventful recovery after cholecystectomy, while those with impaired hepatic function had a turbulent postoperative course or died of hepatic insufficiency, according to the degree of damage to the liver. Pohle and Stewart (41), using the cephalin-cholesterol flocculation test, found 3 mildly positive reactions in 30 patients with chronic cholecystitis with or without gallstones, while 8 of 11 patients with a stone in the common duct gave positive reactions. Recently Mateer and co-workers (42) found impaired hepatic function in over 50 per cent of 67 patients with proved cholelithiasis by use of the intravenous hippuric acid, the cephalin-cholesterol and the colloidal gold test.

The controversial evidence presented by pathologists and by investigators studying hepatic function in cholecystic disease instigated a further study of the incidence of hepatic involvement in disease of the gallbladder.

METHOD OF STUDY

The serum colloidal gold test was performed as described previously in this section. Studies were made on 100 patients with disease of the gallbladder confirmed by roentgenogram or surgical intervention. Ninety-seven of these patients had gallstones proved by one or both of these methods. There were 92 women and 8 men; their ages varied between 20 and 70 years, with an average of 43 years. A careful history was taken to determine the duration of the disease, the degree and duration of jaundice, previous episodes of jaundice and other symptoms, and the presence of fever and chills. Special inquiry was made into such predisposing factors to disease of the liver as the use of drugs (cinchophen, arsenicals, etc.) and previous catarrhal jaundice, syphilis, alcoholism, vitamin deficiencies, and so on.

The 100 patients with proved disease of the gallbladder were classified into four groups according, first, to the past or present history of jaundice (or an elevated serum bilirubin determination) and, secondly, to the clinical evidence of infection of the gallbladder, such as fever, chills, or leukocytosis. These four distinct groups, comprised (1) patients having jaundice with infection, (2) those having jaundice without infection, (3) those having infection without jaundice, and (4) those having quiescent disease of the gallbladder (no jaundice or infection). The term "infection" is used in the clinical sense, to denote a history of fever or chills at any time in the disease process or the presence of leukocytosis or fever. Care was taken to exclude patients with inadequate histories or insufficient hospital studies. The first two groups had stones in the common duct with jaundice and the last two groups had stones in the cystic duct or within the gallbladder. The fourth group, classified as having quiescent disease of the gallbladder, gave no history of previous jaundice or chills and fever. Determinations of the serum bilirubin were normal, and there was no fever or leukocytosis during an adequate period of study. Some of the patients complained of vague abdominal distress, while others were asymptomatic, and the discovery of gallstones on roentgen examination was an incidental finding in many instances.

RESULTS

A positive reaction to the colloidal gold test, indicating hepatic disease, was found in 46 of 100 patients with disease of the gallbladder (Table 3). There were 51 patients who were jaundiced or presented clinical evidence of acute cholecystitis, that is, fever and leukocytosis. Evidence of disease of the liver was found in 55 per cent of these patients. The incidence was highest in the patients with jaundice and infection. Ten of the 17 patients (58.8 per cent) in this group gave a positive reaction to the colloidal gold test. Jaundiced patients without clinical evidence of infection of the gallbladder showed a slightly lower incidence of damage to the liver, 10 of the 19 patients (52.5 per cent) giving a positive reaction to the colloidal gold test. Patients with acute cholecystitis (fever, chills, and leukocytosis) and no jaundice revealed the same incidence of hepatic involvement as those with jaundice and no clinical evidence of infection. In this group 8 of the 15 patients (53.3 per cent) presented a positive reaction to the colloidal gold test.

The incidence of hepatic damage was definitely lower in the group of patients with quiescent disease of the gallbladder. Only 18 of the 49 patients (36.7 per cent) in this group had hepatic involvement as determined by the colloidal gold test, in contrast to over 50 per cent in the groups with jaundice or infection.

It is evident that jaundice or infection of the gallbladder or both were associated with disease of the liver in a large number of patients. Since the duration of the jaundice or infection and the repeated exacerbations and recurrences of the disease over a period of years might be important factors in producing hepatic damage, the incidence of liver disease in relation to the duration of symptoms was studied. Although a further subdivision of these studies resulted in a relatively small number of cases in each group, there was sufficient evidence to indicate that the duration of the disease of the biliary tract was an important factor in producing hepatic damage.

The incidence of disease of the liver increased with the duration of the symptoms in the 17 patients with jaundice and infection of the gallbladder (Table 3). Hepatic damage was demonstrable in 37.5 per cent of patients whose symptoms were of one

week's duration or less, in 50 per cent of patients with symptoms of five weeks' duration, and in 100 per cent of patients with jaundice and infection of more than two months' duration.

The same increase in the incidence of hepatic damage with time was noted in the 19 patients with jaundice but without clinical or laboratory evidence of infection of the gallbladder (Table 3). The patients with intermittent jaundice of one month's duration without concomitant evidence of infection of the gallbladder revealed no incidence of hepatic damage; intermittent jaundice over a period of one to five years caused hepatic involvement in 63 per cent of the patients, while similar symptoms for five to ten years increased the incidence to 75 per cent.

Repeated exacerbations of acute cholecystitis without jaundice increased the incidence of hepatic disease from 33 per cent at the end of one week to 50 per cent at two months and 66.6 per cent at three years (Table 3).

Although 36.7 per cent of the 49 patients with quiescent disease of the gallbladder had a positive reaction to the colloidal gold test, the highest incidence of hepatic involvement occurred in the patients with the shortest duration of symptoms. In contrast to the groups with jaundice or infection, these patients showed a decrease in the incidence of hepatic damage as the period of quiescent disease of the gallbladder increased. The reaction to the colloidal gold test was positive in 11 of the 18 patients (61.1 per cent) with a history of six to twelve months' duration but indicated hepatic disease in only 4 of the 16 patients (25 per cent) whose history was of two to five years' duration and in 3 of the 15 patients (20 per cent) whose history extended over five years.

There appeared to be a correlation between the bacterial flora of the gallbladder, the pathologic changes in the gallbladder, and the incidence of disease of the liver as detected by the colloidal gold test (Table 4). The gallbladder and bile of 22 patients with disease of the gallbladder were cultured. Growth was obtained in 14 of 22 instances. This consisted of Bacillus coli, which was the most prevalent organism, Pneumococcus type III, Streptococcus haemolyticus, Streptococcus viridans, Staphylococcus albus, Pseudomonas pyocyanea, Bacillus typhosus, Bacillus welchii, and diphtheroids.

The reaction to the colloidal gold test was positive in 7

of the 14 patients (50 per cent) whose cultures were positive, in contrast to 2 of the 8 patients (25 per cent) whose gallbladder or bile yielded no bacteria. It is interesting to observe that the more virulent pathogenic organisms were isolated from the 7 patients with a positive reaction to the colloidal gold test, while such bacteria as Streptococcus viridans, Staphylococcus albus, and diphtheroids were cultured from the biliary tracts of the 7 patients with no evidence of disease of the liver. Moreover, the pathologic changes in the gallbladder, both gross and microscopic, were more severe and extensive in the 7 patients with both a positive reaction to the colloidal gold test and virulent pathogenic organisms than in the 7 patients with a negative reaction to the colloidal gold test and less virulent bacteria (Table 5).

COMMENT

The injurious effect on the liver of recurrent jaundice and infection of the gallbladder is readily demonstrable in Table 3. Although the incidence of a positive reaction to the colloidal gold test was highest in the group of patients with both jaundice and infection (58.8 per cent), hepatic damage was equally prevalent in the patients with jaundice alone (52.5 per cent) or with infection of the gallbladder without jaundice (53.3 per cent). The danger of delaying surgical intervention too long is emphasized by the fact that the increase in the incidence of hepatic damage parallels the duration of the disease. Repeated insults to the liver from recurrent jaundice, infection of the gallbladder, or both increased the incidence of disease of the liver to 75 per cent, 66.6 per cent, and 100 per cent, respectively, as the duration of the disease was prolonged.

Jaundice and infection together appear to cause hepatic damage more rapidly than jaundice or infection alone, a pronounced increase in the incidence of hepatic disease occurring within a few weeks. Severe infection within the gallbladder likewise produces rapid hepatic damage, as seen in patient T.S. (Table 5, Patient 9), who complained of recurrent chills and fever of one month's duration. The reaction to the colloidal gold test was positive, and empyema of the gallbladder with gallstones was found at operation. Four similar instances of empyema of the gallbladder

were observed. The patients all showed a positive reaction to the colloidal gold test.

The incidence of hepatic damage was considerably lower (36.7 per cent) in the 49 patients with gallstones and quiescent disease of the gallbladder without jaundice or clinical evidence of infection of the gallbladder. Although an infection of the gallbladder is always present in patients with gallstones, the process in patients without clinical evidence of infection is milder, and the pathologic changes are much less marked, than those observed in patients who manifest such evidence of infection (fever or leukocytosis) or jaundice. The highest incidence of disease of the liver (61.1 per cent) occurs during the more acute stages of the disease in these patients. The infection within the gallbladder then becomes quiescent and apparently mild, and as the period of inactivity increases the regenerative power of the liver is adequate to reduce the incidence of hepatic disease to about 20 per cent in the absence of any recurrent acute infection or jaundice.

The mild pathologic changes observed characteristically on microscopic examination of the gallbladders of patients with quiescent disease of the gallbladder of several years' duration were illustrated in 3 patients with vague abdominal distress and pain in the right upper quadrant of ten to twenty years' duration (Table 5, Patients 8, 13, and 14). Sterile cultures of the bile and gallbladder and negative reactions to the colloidal gold test were observed. At operation gallstones were found and the liver appeared normal.

Recurrent distress of the gallbladder of short duration without jaundice, fever, or leukocytosis is associated with more acute changes within the gallbladder and a higher incidence of hepatic disease. An example of this was seen in patient L.H. (Table 5, Patient 15), who complained of recurrent mild colic of the gallbladder of nine months' duration. There was no evidence of jaundice, fever, or leukocytosis. Gallstones were found at operation, and microscopic study of the gallbladder revealed an extensive round cell infiltration, hemorrhages, and erosions. Streptococcus viridans was cultured from the bile, and the reaction to the colloidal gold test was positive.

Recurrent infection of the biliary tract and jaundice of sufficient duration may result in cirrhosis of the liver. This

was observed in 3 patients (Table 5, Patients 1, 2, and 6) with gallstones and recurrent fever, chills, and jaundice of six to ten years' duration. The reaction to the colloidal gold test was strongly positive in all 3 patients, and microscopic studies of the liver revealed an extensive biliary cirrhosis in 2 (Patients 1 and 6) and an early fibrosis with fatty infiltration in the third (Patient 2). Severe inflammatory changes were observed in the gallbladders of all 3. It is interesting to observe that Streptococcus haemolyticus was cultured from the gallbladders of 2 of the 3 patients and Bacillus coli, Pseudomonas pyocyanea, and Streptococcus viridans from that of the third. Other organisms found were type III pneumococcus and Staphylococcus aureus.

The rapidity with which hepatic damage may be produced was demonstrated in a patient with mild diabetes (Table 5, Patient 3) who had colic of the gallbladder with jaundice and fever for ten days. The reaction to the colloidal gold test was positive ten days after the onset of symptoms. Two months later the gallbladder was removed; the reaction to the colloidal gold test had been positive repeatedly during these two months. Gangrenous cholecystitis was found at operation, and a biopsy of the liver revealed marked fatty infiltration, and vacuolation of the liver cells.

Carcinoma of the gallbladder is another complication of gallstones to be considered in the management of disease of the gallbladder. Adenocarcinoma of the gallbladder was found in 2 patients (Table 5, Patients 11 and 12) with cholelithiasis, in 1 of whom (Patient 11) the tumor had invaded the liver. The reaction to the colloidal gold test was positive in this patient and negative in the one in whom no metastases to the liver were observed (Patient 12). One patient with cholesterosis of the liver (Patient 10) also had a positive reaction to the colloidal gold test.

Ravdin (43), Portis (44), and others have emphasized the importance of evaluating hepatic insufficiency in the management of patients with disease of the gallbladder. The newer and more sensitive methods of detecting early disease of the liver should prove an additional guide in the preoperative and postoperative care of patients with disease of the biliary tract.

SUMMARY AND CONCLUSIONS

1. The colloidal gold test of hepatic function was performed on 100 patients with proved disease of the gallbladder. A positive reaction, indicating hepatic disease, was found in 46 per cent.

2. The incidence of disease of the liver was highest in the patients with jaundice and infection (58.8 per cent) and lowest in the patients with quiescent disease of the gallbladder (36.7 per cent) who presented no history or evidence of fever or jaundice.

3. The incidence of disease of the liver increased with the duration of infection of the gallbladder or jaundice but decreased with time in the group with quiescent disease of the gallbladder.

4. A correlation was noted between the bacterial flora of the gallbladder, the pathologic changes in the gallbladder and the incidence of hepatic disease.

5. Cirrhosis of the liver was found in 3 and carcinoma of the gallbladder in 2 of the 100 patients studied.

6. The importance of detecting early hepatic damage in the management of disease of the gallbladder is discussed.

C. LIVER FUNCTION STUDIES IN DIABETES MELLITUS

INTRODUCTION

The liver is intimately associated with the storage of glycogen, the maintenance of the normal blood sugar level and the formation of ketone bodies. The important role of the liver in carbohydrate metabolism makes the investigation of liver function in diabetes mellitus pertinent. The present section is concerned with the study of the serum colloidal gold reaction in 247 patients with diabetes mellitus.

METHOD OF STUDY

The 247 patients with diabetes were divided into two groups. Group A consisted of 99 patients who had been under careful supervision for the management of their diabetes in a private

institution for several years. Their diet, insulin requirement, and general care were subject to fairly careful control. Group B was comprised of 148 patients receiving treatment for diabetes in a charity institution. The economic, social, and intellectual status of this group made dietary control and insulin treatment more difficult. Clinic visits were less frequent in Group B than in Group A, and the general care of these patients regarding infections, vitamin intake, and control of the blood sugar levels was not equal to that of Group A.

The incidence of liver disease in the obese and non-obese patients was studied in both groups. An increase in weight of more than 10 per cent over the ideal weight was considered evidence of obesity. Observations in the age groups of patients under 35 and over 35 years of age were also made.

The frequency of positive liver tests in mild and severe diabetes was investigated. Mild diabetes was defined arbitrarily by the insulin requirement of less than 25 units of insulin daily. The 247 patients were divided into 2 groups. Those requiring less than 25 units of insulin daily were placed in the classification of mild diabetes, and the others requiring 25 or more units of insulin daily were defined as severe diabetics.

The relationship of liver disease to the proper control of the diabetes was determined. The factors noted in evaluating the control of the diabetes were the level of hyperglycemia, glycosurea, acidosis, hypoglycemia, infections and other complications, insulin resistance, and rapid fluctuations in insulin requirement. The incidence of positive liver tests in patients with acidosis or coma was studied also.

RESULTS

The serum colloidal gold reaction was positive in 91 of the 247 patients with diabetes, an incidence of 36.8 per cent (Table 6). Positive reactions occurred more frequently in the 148 patients of Group B (43.2 per cent) than in the 99 patients of Group A (27.1 per cent).

The test was positive in 28 of the 66 obese patients (42.4 per cent) as compared with 63 of the 181 patients (34.8 per cent) in the non-obese group (Table 7). The incidence appeared to be higher in Group B of both the obese (59.2 per cent) and

non-obese patients (39.6 per cent) than in the corresponding patients of Group A in which positive results were observed in 30 per cent and 25 per cent respectively.

There appeared very little difference in the incidence of liver involvement in patients under 35 years of age (43.4 per cent) than in the older age group (34.8 per cent). Here again the percentage was higher in Group B (48.4 per cent and 41.7 per cent) than in Group A (30.7 per cent and 25.5 per cent) (Table 8).

Hepatic disease appeared to be much more prevalent in severe diabetes than in mild diabetes. The colloidal gold reaction was positive in 62 of 123 patients with severe diabetes (49.9 per cent) compared with 29 of 124 patients with mild diabetes (23.3 per cent) (Table 9). Positive tests were observed with almost equal frequency among the patients of Group A and Group B, with severe diabetes occurring in 47.2 per cent of the former and 52.3 per cent of the latter. Among the mildly diabetic patients, however, the incidence was higher in Group B (30.6 per cent) than in Group A (16.1 per cent).

The highest incidence of liver involvement occurred in the poorly controlled diabetic patients (Table 10). The colloidal gold test was positive in 48 of 84 patients (57.1 per cent) with essentially the same incidence in Group A (54 per cent) and Group B (58.6 per cent). This is to be contrasted with the occurrence of hepatic disease in only 43 of 163 patients (26.3 per cent) with well controlled diabetes (Table 10). Among these patients positive tests were more prevalent in Group B (33.3 per cent) than in Group A (17.7 per cent).

Twenty-two patients were in diabetic coma or acidosis when their liver function was studied. In seventeen of these, or 77.2 per cent, positive colloidal gold reactions were observed.

COMMENT

It has been known for a long time that the diabetic patient is susceptible to fatty infiltration of the liver (45, 46) and to depletion of the liver glycogen (47, 48). It was not surprising, therefore, to find evidence of liver involvement in 36.8 per cent of the 247 diabetic patients studied. This incidence of hepatic disease was somewhat higher than that reported by Meyer (49), who observed impaired liver function in 28 per cent

of 100 diabetics, and by Rabinowitch (50) who noted excess quantities of serum bilirubin in 27.4 per cent of his diabetic patients.

Newburgh and his associates have described a syndrome of hyperglycemia, glycosurea, and a decreased glucose tolerance occurring in obese individuals and resulting presumably from fatty infiltration of the liver (51). A higher incidence of liver involvement in obese than in non-obese patients could be anticipated from his studies. However, there was no significant statistical difference in the incidence of positive colloidal gold reactions in the 66 obese and 181 non-obese diabetic patients reported in this section (Table 7). This may be explained by Newburgh's observation that the "glucose tolerance is unimpaired until the obesity has existed for more than eleven years" (51a). Since many of the patients reported in Table 2 had not been obese for more than 5-6 years, not enough time had elapsed, apparently, to produce a fatty infiltration of the liver.

Although diabetes is usually more severe in the young than in the aged, there was no significant statistical difference in the incidence of hepatic involvement in the two groups (Table 8). The greater regenerative powers of the liver in the young (48) and the shorter duration of the disease compensated for the increased severity of the diabetes.

There was a most striking difference in the incidence of positive colloidal gold reactions in the 124 patients with mild diabetes when compared to the 123 patients with severe diabetes. The liver test was positive in 23.3 per cent of the mild diabetes and in 49.9 per cent of the severe diabetics, an increase of over 100 per cent (Table 9).

The high incidence of positive liver tests in severe diabetes is the result of fatty infiltration of the liver which is a common occurrence in this disease. The extreme degree of fatty infiltration of the liver occurring in depancreatized dogs is well known (52), and the hepatomegaly commonly observed in juvenile and adult diabetics represents fatty infiltration of the liver in most instances.

Failure to control the diabetic state properly results in a considerable increase in the incidence of liver disease. The effects of poor diabetic control on the liver have been observed by White (46) who reported the common occurrence of fatty infiltration of the liver in poorly treated diabetic children. The liver

decreased in size after the administration of insulin and the institution of diabetic management. Failure to control the diabetes in experimental animals, moreover, has resulted in marked fatty infiltration of the liver (53).

The frequency of hepatic involvement in the 247 diabetic patients under discussion was increased from 26.3 per cent, in the well controlled diabetics to 57.1 per cent in the poorly controlled group (Table 10). This represents an increase of more than 100 per cent in the poorly controlled diabetics and emphasizes the importance of the proper control of this disease.

In reviewing Tables 6 to 8 it was observed that the patients in Group B consistently demonstrated a higher incidence of liver involvement than those in Group A. Positive tests were noted in 16.1 to 29.2 per cent more patients in Group B. The determining factors in this discrepancy between the two groups were the lapses of insulin therapy and the lack of general care in regard to the diet and infections. Since the patients in this group were at a lower intellectual and economic level than those in Group A, differences in dietary habits and the intelligent use of insulin were important considerations. This was confirmed by the fact that in the poorly controlled diabetics (Table 10) and in the severe diabetics (Table 9) the incidence of positive liver tests was essentially the same in both groups.

An exceedingly high incidence of hepatic disease was observed in 22 patients with acidosis or coma. Positive colloidal gold reactions occurred in 17 instances, or 77.2 per cent. In liver disease there appears to be a disturbance in the functional capacity of the liver cells to deposit glycogen. This can be demonstrated by the use of hepatotoxic agents and has been observed in association with diseases of the liver (54). When the liver reserves of glycogen are depleted, ketonemia is more likely to occur (55). There is a definite relationship between hepatic disease, glycogen reserve, and ketosis (56). The importance of a high carbohydrate diet in this group is quite evident.

These studies of the colloidal gold reaction in diabetes support the views of Soskin (57) and others that there is a close relationship between hepatic disease and diabetes. It is generally recognized that the glycogen stores of the liver have an important bearing upon its functional capacity and upon its resistance to hepatotoxic agents. In diabetes the glycogen stores of

the liver are frequently depleted and are replaced by fat, particularly in the severe manifestations of the disease. The high incidence of positive colloidal gold reactions in these cases emphasizes the importance of liver disease in severe diabetes.

The liver, moreover, is the principle organ for the homeostatic regulation of the blood sugar level. Soskin and his associates have noted that whenever the blood sugar tends to rise above the normal level, the liver responds by diminishing its output of sugar into the blood. Insulin is an important factor in the endocrine balance which controls the level at which the liver regulates the blood sugar, but the intrinsic homeostatic regulating mechanism of the liver is thought to be the most important factor of all (57).

When the liver is abnormal, the mechanism by which it regulates the blood sugar is greatly impaired, and its response to insulin is diminished. The detection of liver disease in approximately 50 per cent of patients with severe diabetes may be of considerable significance in the dietary and medical management of the disease.

SUMMARY

1. The serum colloidal gold reaction was positive in 91 of 247 patients with diabetes, an incidence of 36.8 per cent. Liver involvement was observed more frequently in the 148 patients receiving irregular care (43.2 per cent) than in the 99 patients under constant supervision (27.1 per cent).

2. The reaction was positive in 62 of 123 patients with severe diabetes (49.9 per cent) in contrast to 29 of 124 patients (23.3 per cent) with mild diabetes.

3. The frequency of hepatic involvement was increased from 26.3 per cent in the well controlled diabetics to 57.1 per cent in the poorly controlled group.

4. Positive reactions were observed in 28 of 66 obese patients (42.4 per cent) as compared with 63 of the 181 non-obese patients (34.8 per cent).

5. The incidence of liver involvement was 43.4 per cent in the patients under 35 years of age and 34.8 per cent in the older age group.

CONCLUSIONS

1. Diabetes mellitus is associated frequently with disease of the liver as is evidenced by the presence of a positive serum colloidal gold reaction.

2. Hepatic involvement is much more prevalent in severe than in mild diabetes.

3. Failure to control the diabetic state properly results in a considerable increase in liver disease as is seen particularly in patients with acidosis or coma.

4. There is no significant statistical difference in the frequency of liver involvement in the younger and older age groups, and in obese and non-obese patients.

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TABLE 1

STUDIES ON HEPATIC FUNCTION IN HEPATOLENTICULAR DEGENERATION

Case	Patient	Plasma Bilirubin, Mg. Per Cent	Plasma Albumin/ Globulin, Gm. Per Cent	Plasma Cholesterol/ Cholesterol Esters, Mg. Per Cent	Galactose Tolerance	Bromsulphalein Retention
1	S.R.	1.51	4.27/2.58	185/81	1.2	Normal
2	J.S.	0.89 0.79	4.28/2.84	145/56	0.0	Normal
3	S.R.	0.73	3.49/3.01	202/96 (1935) (1940)	2.5 1.2	Normal (1935) 10% 1940)
4	J.S.	0.63	4.02/2.32	201/75	....	Normal
5	N.D.	0.17	4.82/2.12 4.64/2.17	173/80 179/76	0.53	Normal
6	R.M.	....	4.35/2.48	153/54	....	.....
7	R.W.	0.86	3.90/2.90	168/67	....	100%
8	L.W.	0.85	4.31/1.73	.....	0.60	Normal
9	H.W.	....	.....	.....	....	.....

TABLE 2

TESTS OF HEPATIC FUNCTION IN HEPATOLENTICULAR DEGENERAGION

Case	Patient	Dextrose Tolerance, Capillary Blood Sugar; Mg. Per Cent; Hours After Injection							Hippuric Acid Excretion; Gm. of Benzoic Acid Excreted	Bilirubin Excretion, Percentage Retained	Prothrombin Time, Percentage of Normal	Serum Colloidal Gold Test
		0	1/2	1	2	3	4	5				
1	S.R.	92	171	231	261	72	77	93	2.80	12.9	48	532
											46	522
2	J.S.	93	210	182	150	58	..	..	....	2.1	..	532
		93	166	205	135	69	76	80	3.31	10.4	68	532
3	S.R.	103	138	138	240	165	86	70	3.04	6.6	72	522
											70	532
											73	521
4	J.S.	78	142	236	162	160	..	..	2.24	0	65	522
		72	193	246	207	134	..	..	....	....	65	532
5	N.D.	67	166	113	...	57	..	72	3.66	16.2	95	522
		76	186	167	93	58	75	73	....	....	..	521
6	R.M.	..	...	...	...	...	..	..	....	....	..	522
												521
7	R.W.	100	179	181	...	...	..	..	1.03	1.1	65	532
												522
8	L.W.	83	151	174	135	96	69	98	2.70	13.8	48	222
												222
9	H.W.	...	...	...	...	...	..	..	....	....	100	532
												522

TABLE 3

INCIDENCE OF POSITIVE REACTIONS TO THE COLLOIDAL GOLD TEST IN PATIENTS WITH DISEASE OF THE GALLBLADDER

	Number of Patients	Incidence of Positive Reactions to Colloidal Gold Test			
		No.	%	No.	%
A. Jaundice with infection.....	17			10	58.8
1. Duration of symptoms					
(a) 1 to 7 days.....	8	3	37.5		
(b) 5 weeks.....	4	2	50.0		
(c) 2 or more months.....	5	5	100.0		
B. Jaundice without infection....	19			10	52.5
1. Duration of symptoms					
(a) 1 month.....	4	0			
(b) 1 to 5 years.....	11	7	63.0		
(c) 5 to 10 years.....	4	3	75.0		
C. Infection without jaundice....	15			8	53.3
1. Duration of symptoms					
(a) 1 week.....	3	1	33.3		
(b) 1 to 2 months.....	6	3	50.0		
(c) 1 to 3 years.....	8	4	66.6		
D. Quiescent disease of gallbladder (no fever or jaundice)....	49			18	36.7
1. Duration of symptoms					
(a) 6 months to 1 year.....	18	11	61.1		
(b) 2 to 5 years.....	16	4	25.0		
(c) 5 or more years.....	15	3	20.0		
Total patients studied.....	100			46	46.0

TABLE 4

BACTERIAL FLORA OF THE GALLBLADDER AND BILE

	Number of Patients	Incidence of Positive Reactions to Colloidal Gold Test	
		No.	%
A. Growth.....	14	7	50
B. coli..... 5			
Pneumococcus of type III 1			
Str. haemolyticus..... 2			
Str. viridans..... 1			
Staph. albus..... 1			
Ps. pyocyanea..... 1			
B. typhosus..... 1			
B. welchii..... 1			
Diphtheroids..... 1			
B. No bacterial growth.....	8	2	25

TABLE 5

SYMPTOMATOLOGY, BACTERIOLOGY, AND PATHOLOGY OF DISEASE OF THE GALLBLADDER

Patient	Symptoms	Duration	Bacterial Flora of Gallbladder and Bile	Pathologic Changes in Gallbladder	Pathologic Changes in Liver	Reaction to Colloidal Gold
1. A.G.	Recurrent chills, fever and jaundice	6 yr.	B. coli, pneumococcus of type III, Str. haemolyticus	Extensive fibrous and round cell infiltration; chronic cholecystitis and cholelithiasis	Cholangitis and biliary cirrhosis (autopsy)	Strongly positive
2. B.V.	Recurrent chills, fever and jaundice with colic	10 yr.	B. coli, Pseudomonas pyocyanea, Str. viridans	Round cell infiltration, erosions and hypertrophy; chronic cholecystitis and cholelithiasis	Periductal and intralobular round cell infiltration; early fibrosis, fatty infiltration	Strongly positive
3. R.B.	Chills, fever, jaundice, colic (mild diabetes mellitus and obesity)	10 days	B. coli	Gangrenous cholecystitis; cholelithiasis	Marked fatty infiltration; mild lymphocytic infiltration; vacuolation of liver cells	Positive
4. S.P.	Recurrent colic, chills and fever; no history of jaundice	10 mo.	No growth	Acute cholecystitis, edema and thickening of gallbladder wall, hemorrhages, round cell infiltration and denuded mucosa; cholelithiasis	.....	Positive
5. A.B.	Recurrent colic; no fever, chills or jaundice	6 mo.	No growth	Thickened muscularis, round cell infiltration; cholelithiasis	.....	Positive

TABLE 5--Continued

Patient	Symptoms	Duration	Bacterial Flora of Gallbladder and Bile	Pathologic Changes in Gallbladder	Pathologic Changes in Liver	Reaction to Colloidal Gold
6. T.M.	Recurrent colic, fever, chills and jaundice	6 yr.	Str. haemolyticus, Staph. aureus, Str. viridans	Chronic cholecystitis and cholelithiasis; cicatricial stenosis of common duct	Biliary cirrhosis of liver	Strongly positive
7. B.K.	A. Colic, chills, fever and jaundice	3 wk.	B. coli, Str. viridans	Minimal round cell infiltration, small erosions, fibrosis of walls of gallbladder; cholelithiasis	.....	Negative
	B. Postoperative colic, jaundice and fever	5 wk.	B. coli	Choledocholithiasis; dilatation of common duct	.....	Positive
8. C.R.	Pain in right upper quadrant; no jaundice, fever or chills	10 yr.	No growth	Minimal round cell infiltration and fibrosis; one stone in cystic duct of gallbladder	.....	Negative
9. T.S.	Recurrent fever and chills	1 mo.	.....	Empyema of gallbladder and cholelithiasis	.....	Positive
10. D.S.	Recurrent colic, jaundice, fever and chills	5 yr.	.....	Cholelithiasis; large stone in common duct; dilated common and cystic ducts	Nodules in liver suggesting biliary cholesterosis; dilated hepatic ducts	Positive

TABLE 5--Continued

Patient	Symptoms	Duration	Bacterial Flora of Gallbladder and Bile	Pathologic Changes in Gallbladder	Pathologic Changes in Liver	Reaction to Colloidal Gold
11. A.G.	Recurrent colic; no fever, chills or jaundice	4 mo.	.....	Cholelithiasis; adenocarcinoma of gallbladder	Adenocarcinoma of liver adjacent to gallbladder	Positive
12. A.M.	Recurrent epigastric pain; no jaundice, fever or chills	5 mo.	No growth	Adenocarcinoma of gallbladder; cholelithiasis; round cell infiltration and fibrosis	No metastases to liver	Negative
13. C.D.	Vague abdominal distress; occasional pain in right upper quadrant	10 yr.	No growth	Minimal cellular infiltration, small mucosal erosions, cholelithiasis	.....	Negative
14. A.K.	Vague abdominal distress	20 yr.	No growth	Minimal round cell infiltration, fibrosis, cholelithiasis	.....	Negative
15. L.H.	Recurrent colic; no jaundice or fever	9 mo.	Str. viridans	Marked round cell infiltration, hemorrhages, mucosal erosions; cholelithiasis	.....	Positive

TABLE 6

INCIDENCE OF LIVER DISEASE IN DIABETES

	Number of Patients	Positive Serum Colloidal Gold Reactions
Group A	99	27 (27.1%)
Group B	148	64 (43.2%)
Total	247	91 (36.8%)

TABLE 7

INCIDENCE OF LIVER DISEASE IN OBESE AND NON-OBESE DIABETIC PATIENTS

	Obese Patients	Positive Liver Tests	Non-Obese Patients	Positive Liver Tests
Group A	39	12 (30.0%)	60	15 (25.0%)
Group B	27	16 (59.2%)	121	48 (39.6%)
Total	66	28 (42.4%)	181	63 (34.8%)

TABLE 8

THE RELATION OF AGE TO POSITIVE LIVER TESTS IN DIABETIC PATIENTS

	Number of Patients	Positive Serum Colloidal Gold Reactions
Under 35		
Group A	13	4 (30.7%)
Group B	33	16 (48.4%)
Total	46	20 (43.4%)
Over 35		
Group A	86	22 (25.5%)
Group B	115	48 (41.7%)
Total	201	70 (34.8%)

TABLE 9

INCIDENCE OF POSITIVE LIVER TESTS IN MILD AND SEVERE DIABETES

	Number of Patients	Incidence of Positive Colloidal Gold Reaction
Mild Diabetes		
Group A	62	10 (16.1%)
Group B	62	19 (30.6%)
Total	124	29 (23.3%)
Severe Diabetes		
Group A	37	17 (47.2%)
Group B	86	45 (52.3%)
Total	123	62 (49.9%)

TABLE 10

POSITIVE LIVER TESTS IN WELL CONTROLLED AND POORLY CONTROLLED DIABETES

	Number of Patients	Incidence of Positive Colloidal Gold Reaction
Well Controlled		
Group A	73	13 (17.7%)
Group B	90	30 (33.3%)
Total	163	43 (26.3%)
Poorly Controlled		
Group A	26	14 (54.0%)
Group B	58	34 (58.6%)
Total	84	48 (57.1%)

PART V. SUMMARY

SUMMARY

Studies of the plasma proteins of 96 patients with definite liver disease revealed a characteristic increase in the globulin and decrease in the albumin content in many instances. The most marked albumin-globulin shifts occurred in cirrhosis of the liver and were associated with advanced hepatic destruction. The least marked alterations of the plasma proteins were found in the acute parenchymatous diseases and in neoplastic involvement of the liver.

In view of the fact that the cerebrospinal fluid flocculated colloidal gold suspensions presumably by virtue of its increased globulin and decreased albumin content, studies were undertaken to determine the effect of the blood sera of patients with hepatic disease upon the suspension stability of colloidal gold solutions.

The addition of a properly acidified colloidal gold suspension to serial dilutions of sera from patients with liver disease resulted in the flocculation of the colloidal gold and produced a paretic type of curve similar to that obtained with the spinal fluid. Flocculation of the colloidal gold occurred rarely with the sera of normal individuals and patients with extrahepatic diseases.

The colloidal gold reaction of the blood serum was then studied in 96 cases of different types of liver disease, confirmed by autopsy, biopsy, or laparotomy in many instances and was found to be a sensitive indicator of liver disease. The reaction was positive in 92.7 per cent of these cases and was particularly sensitive in cirrhosis of the liver and in the acute parenchymatous diseases. In several instances the colloidal gold reaction detected liver disease, later confirmed by operation or autopsy, which was not indicated by the other chemical and clinical tests performed. The test was negative, moreover, in 97 per cent of the control group, consisting of 200 normal individuals and 100 patients with various extrahepatic diseases.

The colloidal gold reaction did not depend primarily upon

a quantitative increase in serum globulin or upon a low or inverted albumin-globulin ratio. Positive reactions were obtained in 29 cases of hepatic disease with normal serum globulin determinations and in 21 cases with normal albumin globulin ratios. The reaction remained unaltered in diseases like nephritis or nephrosis in which the serum globulin was elevated or the albumin-globulin ratio inverted. These studies suggested that the serum colloidal gold reaction depended upon a qualitative rather than a quantitative variation in the serum globulin.

In order to investigate further the relationship of hepatic disease to the serum proteins and to study the mechanism of the blood serum colloidal gold reaction in diseases of the liver, the sera of patients with various types of hepatic disease were subjected to electrophoretic analysis. A large increase in the gamma globulin and a decrease in the serum albumin characterized the alteration of the serum proteins in diseases of the liver. The changes were most pronounced and consistent in cirrhosis of the liver and in the acute parenchymatous hepatic diseases. In many instances of the latter the gamma globulin was elevated in spite of normal albumin-globulin values obtained by the usual methods of fractional precipitation. The degree of abnormality of the electrophoretic patterns was related in most instances to the severity of the hepatic disease.

It appeared from these studies that an increase in the gamma globulin component and a decrease in the albumin fraction of the blood serum were responsible for the colloidal gold reaction in liver disease. To study further the mechanism of this reaction, electrophoretically pure globulin fractions were added to the normal blood serum in increasing concentrations, equivalent to the values obtained electrophoretically in liver disease. The serum was then diluted, and the colloidal gold reaction was performed in the usual manner.

The addition of electrophoretically pure gamma globulin to normal serum produced a positive colloidal gold test identical with that obtained with the sera of patients with liver disease. The degree of flocculation increased with the increment in gamma globulin concentration. The addition of pure alpha and beta globulin to normal serum had no effect upon colloidal gold flocculation. Pure albumin inhibited the serum colloidal gold reaction. The addition of albumin to the serum of a patient with

cirrhosis of the liver converted the positive test to negative. In view of the fact that the most characteristic and consistent alteration of the serum proteins in hepatic disease was a decrease in serum albumin and a relatively large increase in gamma globulin, these studies suggested that the mechanism of the colloidal gold reaction in liver disease depended upon a relative increase in the gamma globulin content of the serum.

The positive reactions obtained in certain instances of syphilis, multiple myeloma, lymphopathia venereum, and lupus erythematosis were attributed to the increase in gamma globulin observed occasionally in these diseases. In the infectious diseases, however, the alpha globulin of the serum was increased; the colloidal gold reactions were negative consistently in this group. The uniformly negative reactions in cases of nephritis and nephrosis, associated with increased serum globulin determinations, were explained by the decrease in gamma globulin and the increase in the alpha and beta components of the serum in these diseases.

It was thought advisable to substantiate further the mechanism of colloidal gold flocculation by investigating the effect of the electrophoretically pure proteins upon the spinal fluid colloidal gold reaction. Increasing amounts of the electrophoretically pure albumin and globulin fractions were added, within physiological limits, to normal spinal fluid, and the colloidal gold reaction was performed in the usual manner. The addition of pure gamma globulin to normal spinal fluid produced a typically paretic colloidal gold curve similar to that obtained in the sera of patients with hepatic disease. Alpha and beta globulin did not affect the colloidal gold reaction. Albumin inhibited colloidal gold flocculation. The addition of pure human albumin to the spinal fluid of patients with paresis converted the paretic curve to normal. These observations confirmed the importance of the gamma globulin fraction in the flocculation of colloidal gold suspensions.

The colloidal gold reaction of the spinal fluid and that of the blood serum from the same patient were identical in some instances and differed greatly in others depending upon the disease process and the permeability of the blood brain barrier. The colloidal gold reaction was positive in the blood serum and negative in the spinal fluid of patients with liver disease. In

neurosyphilis, however, the colloidal gold reaction was negative in the blood serum and positive in the spinal fluid, presumably due to the formation of gamma globulin within the tissues of the central nervous system. In the presence of both hepatic cirrhosis and neurosyphilis the colloidal gold reaction was positive in both the serum and the spinal fluid. Negative reactions in both the spinal fluid and blood serum were observed in patients with no evidence of hepatic or central nervous system disease. It appeared from these studies that the relative concentrations of gamma globulin in the serum and spinal fluid were the deciding factors in the colloidal gold reaction.

In view of the sensitivity of the colloidal gold reaction in detecting liver disease it was thought desirable to investigate the clinical application of this reaction to the study of (1) hepatolenticular degeneration, (2) diseases of the gallbladder, and (3) diabetes mellitus.

The study of liver function in hepatolenticular degeneration was of particular interest in that the usual tests of hepatic function had failed to demonstrate liver insufficiency in spite of the extensive cirrhosis of the liver which was described as a constant feature of the disease at autopsy. Nine patients suffering from this rare disease were selected for this study. The chemical investigations included determinations of the serum bilirubin, albumin, globulin, cholesterol, and cholesterol esters. The following liver function tests were performed: galactose tolerance, bromsulphalein retention, five-hour dextrose tolerance, hippuric acid excretion, bilirubin excretion, prothrombin time, and the colloidal gold reaction of the serum. The serum colloidal gold reaction appeared to be the most sensitive test for cirrhosis of the liver demonstrating hepatic disease in 8 of the 9 cases studied. The bilirubin excretion and the prothrombin time were found to be very reliable indicators of hepatic disease revealing liver involvement in 5 to 6 of the 8 cases investigated by these methods. The chemical studies and the bromsulphalein retention test were of little or no value in detecting hepatic disease in these cases, and the hippuric acid test gave positive results in less than half of the cases studied.

In an effort to evaluate the importance of hepatic insufficiency in the medical and surgical management of biliary tract disease, the colloidal gold test was performed on 100 pa-

tients with proved disease of the gallbladder. A positive reaction indicating hepatic disease was found in 46 per cent, the incidence being highest in the patients with jaundice and infection and lowest in the patients with quiescent gallbladder disease. The prevalence of hepatic disease increased with the duration of the gallbladder infection or jaundice, and a correlation was noted between the bacterial flora, the disease process in the gallbladder, and the incidence of hepatic involvement.

The important role of the liver in carbohydrate metabolism suggested the investigation of liver function in diabetes mellitus. The colloidal gold reaction was studied in 247 such patients. Evidence of liver involvement was obtained in 36.8 per cent of this group. Hepatic disease was much more prevalent in severe than in mild diabetes, and patients in acidosis or coma had an extremely high incidence of positive tests. Failure to control the diabetic state resulted in a very appreciable increase in the frequency of liver involvement. The detection of liver disease in severe diabetes was of considerable significance in the dietary and medical management of the disease.

PART VI. CONCLUSIONS

CONCLUSIONS

1. The most characteristic alteration of the serum proteins in liver disease is a large increase in the gamma globulin component and a decrease in the serum albumin fraction. These changes occur most frequently and to the greatest extent in cirrhosis of the liver and next most frequently in the acute parenchymatous diseases.

2. The degree of abnormality of the electrophoretic patterns is related, in most instances, to the severity of the hepatic disease.

3. Serial dilutions of the blood serum of patients with liver disease flocculate colloidal gold suspensions producing a paretic type of curve similar to that obtained with spinal fluid.

4. The flocculation of colloidal gold suspensions rarely occurs with the sera of normal individuals and of patients with extrahepatic diseases.

5. The mechanism of the colloidal gold reaction in liver disease depends upon a relative increase in the gamma globulin of the serum.

(a). Gamma globulin is the only serum globulin fraction which flocculates colloidal gold suspensions.

(b). Pure albumin inhibits colloidal gold flocculation.

(c). The gamma globulin is increased and the albumin is diminished in the sera of patients with liver disease.

6. The blood serum colloidal gold reaction is a sensitive method of detecting liver disease and may reveal hepatic damage not indicated by other chemical and clinical tests.

7. The demonstration of liver involvement in hepatolenticular degeneration, gallbladder disease, and diabetes mellitus is of considerable significance in the medical management of these diseases.